Chemical, Physical and Biological Factors in the Toxic Effects of Leachable Plastic Additives

THESIS SUBMITTED

TO

THE ALIGARH MUSLIM UNIVERSITY, ALIGARH

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN CHEMISTRY

SUMMARY

BY

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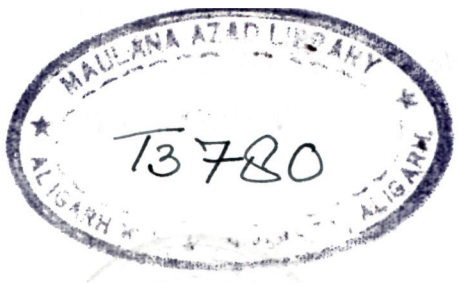
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SUMMARY AND CONCLUSIONS

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Application of plastics is rapidly increasing in our country. Today, it is difficult to conceive a world without plastics which have replaced the convention materials like, wood, steel and glass. Ranging from day-to-day of home appliances, plastics are being used in agricultures, automobiles, space vehicles, buildings and biomedical devices. The biomedical application of plastics includes devices for collecting or administering of fluids (e.g. transfusion sets, catheters, tracheostomy tubes and syringes), storage of blood and solutions for injection, and for prosthetic application (e.g. heart values, blood vessels and joints). Plastics are preferred over the other conventional materials due to their light weight, mould resistance and wide range of colour acceptability. There has been a tremendous increase in the production of plastics during the last two decades. The total plastic production increased from 0.24 lakh tonnes in 1965 to 4.92 lakh tonnes in 1985 and is expected to be around 24.4 lakh tonnes by the end of this century.

Plastics are basically organic polymers, constructed by chain like attachments of individual building blocks called monomers. The commercial plastics in addition to the polymer generally has one or more supplementary agents, referred to as additives. These additives acts as ***plasticizing, stabilizing, filling*** *and* ***antistatic agents, flame retardents etc.*** *to impart desired chemical and physical properties.*

In comparison to other industrial chemicals, plastics are comparatively new and not much is known about their toxicological effects. Rapidly increasing direct and indirect contact of human

body with plastics have attracted the attention of health scientists all over the world on the possible health hazards of these polymers and the chemical additives used in their processing. Finished plastics are generally considered to be safe if they are synthesized under standard conditions using the chemicals recommended by national and international regulatory agencies and used properly. However, the unreacted monomers and copolymer such as vinylchloride, acrylonitrite, styrene and methylmethacrylate and chemical additives such as plasticizer, stabilizer, colourants, u.v. absorbers used in its processing are reported to leachout into the stored commodity under certain conditions and have been a cause of concern due to their toxicity. Migration of chemical additives from finished plastics have become both a scientific and regulatory issue.

The workers involved in the production of various chemical additives, handling and processing of plastics have been found to suffer from diseases like dermatitis, allergic responses, bronchial asthma, acroosteolysis with scleroderma, Raynauds phenomenon, hepatic and nervous disorders and even cancer. These diseases have been attributed to the monomers and chemical additives used in the manufacture of plastics. Besides industrial workers, general population including children and pregnant mothers can also be exposed to certain levels of the chemical additives for prolonged period of time due to their leaching from finished plastics. Migration of chemical additives above the permissible limit may be hazardous for the consumers of plastics on long term basis due to the toxic nature of some of the additives.

Regulatory agencies in different countries had laid down guidelines for the safe use of plastics. In our country, Bureau of Indian standards (BIS) and Central Committee for Food Standards, Ministry of Health and Family Welfare have laid down some guidelines for the testing of plastic materials for use in food and biomedical applications. In most cases the materials stored in plastic pouches/ containers in our country are often subjected to high temperature and sunlight. In rural area and also in some of the urban area plastic containers are used for the storage of food materials which have an acidic pH e.g. pickles, and are exposed to sunlight for curing. Usually fruit juices, vegetable oils, transfusion fluids and medicines are stored in plastic pouches/containers for prolonged period than anticipated and are exposed to high temperature in summer season during delivery. The specifications laid down by BIS, for the safety evaluation of the plastic materials have not taken full account of such specific conditions, prevalent in our country. The leachability of the chemical additives could be modified by physico-chemical factors and may render the materials harmful.

Among the leachable components, plasticizers and stabilizers have assumed a great concern. Significant amount of data on the toxicity of phthalate esters is available in the literature while adequate information on the stabilizers particularly organotin is not available. In order to understand the toxicity of organotin compounds the candidate has selected dibutyltin dilaurate, a commonly used PVC Stabilizers for its studies. During recent years there has been a tremendous increase in the production of organotin compounds with their ever increasing technical application. The annual world

production of organotin compounds have grown rapidly from 400 tonnes in 1950 to 25,000 tonnes in 1975 and is expected to grow over 50,000 tonnes by 1990. Alongwith the rapid growth in the production of organotin compounds, concern about their possible environmental and health effects has also increased and is a subject of concern. The report of their migration from PVC containers into the biological system has aroused a great attention over their toxicological potential.

Since significant amounts of organotins are reported to leach out from plastics, possibility of the exposure of pregnant women and young children to varying quantity of organotin compounds is eminent. Unfortunately no data is available on this aspect of their toxicity.

In the present investigation, the candidate has carried out in depth studies on the effects of certain physico-chemical factors like sunlight, temperature, storage time, pH and the chemical nature of the extracting media on the migration of chemical additives from plastic materials used for the storage, packaging and delivery of food, drinking water and biological fluids (blood, saline and dextrose). During the course of investigation, about 35 different brands of plastic materials obtained from the local market were evaluated to assess the quality of plastics being used commonly.

In order to assess the neurotoxic potential of dibutyltin dilaurate, a leachable stabilizer, its effect on levels of biogenic amines, locomotor activity and learning ability were studied in rats. Attempts were also made to investigate the role of age and sex in the neurotoxic effects of DBTL, since no information was available

on this aspect of the organotin stabilizer.

A. *SAFETY EVALUATION OF PLASTIC MATERIALS BEING INCREASINGLY USED IN THE STORAGE, PACKAGING AND DELIVERY OF FOOD, DRINKING WATER, COSMETICS AND LIFE SAVING FLUIDS.*

About 35 brands of plastic materials (six hundred samples) used for the storage, packaging and delivery of food, drinking water, cosmetics and life saving fluids were subjected to safety evaluation tests for plastics. The chemical test procedure used was based on the recommendations of Bureau of Indian Standards, British Pharmacopoeia, Pharmacopoeia of Japan, Food and Drug Administration (USA), British Plastic Federation and National Formulary (USA) with modifications. The various physico-chemical tests performed were the observations of the colour and odour of extracts, presence and contents of U.V. absorbing materials, oxidisable matters, heavy metals and degree of global (overall) migration. Residue on ignition of plastics and metal contents in the residue of plastics were also estimated. In 20 brands of the plastics, contents of styrene present as unreacted monomer was also estimated. The plastics used in biomedical applications were subjected to the biological tests such as gross toxicity, mortality or any other abnormality as a result of exposure of the plastic extracts to the albino mice which was used as the experimental model.

Out of the 35 brands of the samples tested, 8 brands of plastics showed leaching of colour and 6 showed presence of odour in the extracts indicating that they do not meet the requirements laid down by BIS. Migration of the U.V. absorbing materials was

above the permissible limit in one or more extracts of 21 brands of plastics. Global migration and content of oxidisable matters were also above the permissible levels in 21 and 6 brands of plastics respectively. The contents of heavy metals in extracts obtained from 26 brands of the samples was high. The residue on ignition was above the permissible limit in 30 brands and content of heavy metals in the plastic residue was above the permissible limit in 7 brands of the samples.

Out of 20 brands of the samples tested for residual monomer, only 6 brands showed presence of styrene as unreacted monomer of which only 2 brands showed the contents of the monomer above the permissible limits.

Biological tests performed on the plastics extract were satisfactory. No mortality or signs of gross toxicity were noticed in the animals administered with the plastic extracts in distilled water, saline and ground nut oil orally, intravenously or intraperitoneally.

About 57.00% of the total plastic samples tested did not meet guidelines of BIS and about 98.00% of the samples were unable to meet the requirements laid down by other international regulatory agencies. It is apparent that most of the plastic materials available in the market do not meet the requirements laid down for their safe use. The plastic material being used for packaging of food and life saving fluids should be subjected to critical safety evaluation tests regularly. The manufacturers of the plastic products should ensure before introducing them into the market that the materials manufactured meet the requirements laid down for their safe

use. Proper precautions during the use of plastics are essential and plastics should be used only for those purposes for which they have been designed and tested. The possibility of the health hazards to the consumers of plastic exists, if non-food grade or untested plastics would be used, since some of the leachable additives are toxic.

The acute biological tests performed with the extracts of plastics used in biomedical suggests that these articles are not likely to pose any health hazards. However, the consumers of the plastic may be at risk on the long term basis, due to the migration of chemical additives which are toxic in nature.

B. *EFFECT OF SOME PHYSICO-CHEMICAL FACTORS ON THE MIGRATION OF CHEMICAL ADDITIVES FROM FINISHED PLASTICS.*

Migration of chemical additives (U.V. absorbing materials, heavy metals, oxidisable matters, etc.) from the finished plastics was found to increase with the increase in the acidity and alkalinity of the extracts. It also increased with the increase of extracting temperature and storage time. It was also observed that sunlight accentuates the migration of UV absorbing materials, and heavy metals. Influence of sunlight on the global migration appears insignificant in our experimental conditions, however, it assumes paramount significance as the exposure of plastics are generally perennial, in which case enhancement of the leachability of the chemical additives may occur. Under sunlight, selective migration of Mn occurred from lunch boxes and freeze bottles. The enhanced migration was not related to temperature since it remained unaltered when samples

were kept at the same temperature and duration in a hot air oven. It is difficult to identify the exact factors responsible for such an enhanced rate of migration of UV absorbing materials and heavy metals from the plastic materials under sunlight. Possibly, factors such as UV radiations and oxidants like ozone and other environmental factors may be responsible for such an effect. polymer fragmentation under sunlight and specially under UV rays has been reported.

Out of the eight brands of samples (freeze bottles, water tumblers, lunch boxes and blood bags, each of two brands) studied, six samples showed maximum global (overall) migration in basic medium (pH=10) and two in acidic medium (pH=2.5), in comparison to the global migration obtained in aqueous, alcoholic and saline media. Global migration included the organotin compounds, phthalates, heavy metals and those chemicals which are not volatile upto 90°C. This suggests that migration of chemical additives could be increased with the increase in acidity and basicity. Such an increase may be due to the polarization of bonds of the additives attached with the polymer in the extractants having acidic and basic pH.

Significant amounts of some of the leachable chemical additives of plastics have been detected in the environment and tissues of animals and human beings. The body burden of injurious chemicals in the humans may further be increased as a result of their leaching from the finished plastic products.

The present data suggests that the use of plastic articles other than those for which they have been tested may be hazardous for the consumers, as sunlight, higher temperature, pH of the stored

material enhanced the migration of the injurious chemical additives from finished plastics. Use of plastic utensils or pouches for the storage and packaging of food, drinking water and biological fluids for longer duration is also not advisable as migration of chemical additives also increased significantly with the increase of the duration of extraction. It is also important to underscore at this juncture that the various brands of plastic samples studied, when used for shorter duration of time (upto 24 hours) at room temperature (25°C), do not pose serious threat to the consumers, irrespective of the quality or nature of the food material stored, since migration of chemical additives under such conditions were within the permissible limit.

Our observations are of immense significance as they could serve as a base line data for formulating the guidelines for the safe use of plastics.

C. TOXIC EFFECTS OF DIBUTYLTIN DILAURATE (DBTL)- A LEACHABLE PLASTIC ADDITIVE: NEUROBEHAVIORAL AND BIOCHEMICAL EFFECTS.

Neurotoxicity of lower homologues of organotin compounds are well known. However, adequate information about the toxicity produced by higher homologues of organotin compounds are not known. The industrial workers and the general population including the pregnant mothers are exposed to varying amounts of the organotin compounds for prolonged period of time either at the work place, or due to their leaching from plastic materials. Since the growing children may also be exposed to these stabilizer due to their leaching from

pacifier, baby feeding bottles, transfusion pouches etc., role of age and sex was studied in the neurotoxicity of higher homologues of organotin compounds using some behavioral and biochemical parameters. Weanling, juvenile and adult male and female albino rats exposed to 20, 40 and 80 mg/kg DBTL, orally for 3 consecutive days were found to be lethargic, dull and weak throughout the experimental period in comparison to controls. The animals exposed to 40 and 80 mg DBTL/kg also showed swelling and reddening around the mouth area associated with brown pigmentation on the central body surface and hindlimb weakness.

A gradual loss in the body weight of DBTL exposed rats was observed in weanling, juvenile and adult rats in comparison to the age matched controls in a dose dependent manner, which was significant in the animals exposed to 40 and 80 mg DBTL/kg. Juvenile rats of both sexes were found to exhibit maximum decrease in their body weight in comparison to the other two groups of animals. However, DBTL had no significant effect on the wet weight of the brain (total or relative to body weight) in all groups of animals except the animals exposed to 80 mg/kg, where significant reduction in the brain weight were noticed.

The animals exposed to 20 mg/kg DBTL, showed no mortality in either males or females of weanling and juvenile group, while the rats of adult groups showed 10% mortality in males and 20% mortality in females. Exposure of animals to 40 mg/kg resulted in 10% and 20% mortality in case of weanling and juvenile males and females respectively and 40% and 60% in adult males and females respectively. At the highest level of DBTL treatment (80 mg/kg)

an increased rate of mortality was noticed in all groups. Weanling animals showed 20% and 30% mortality in males and females, the juvenile rats showed 30% mortality in both sexes and the adult animals showed 60% and 90% mortality in males and females respectively.

These results suggest that the DBTL produces a dose dependent toxicity and the female rats of all ages were more susceptible to this chemical.

Effect of DBTL (20, 40 and 80 mg/kg body weight) on the spontaneous and drug-induced motor activity and learning ability was investigated in weanling, juvenile and adult male and female albino rats to see if this chemical induces any functional deficits in rats. All the treated animals were found to exhibit a dose dependent decrease in the learning ability and spontaneous and drug induced motor activity. Female rats of all the groups were found to be more affected by DBTL as compared to the males of the same age. The juvenile group of rats showed maximum decrease in the learning ability and spontaneous and drug induced motor activity in comparison to weanling and adult rats. The minimum decrease in all parameters were noted in weanling rats.

A dose dependent decrease in the retention of memory after seven days of memory aquisition as assessed by conditioned avoidence response in the rats of various groups was observed in all the three age groups. The maximum decrease in the retention of memory was observed in the juvenile male and female rats in comparison to weanling and animals of adult rats.

Effect of DBTL exposure on the levels of norepinephrine (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) were studied

only in the rats of female, juvenile group in the whole brain and different brain areas. It was observed that this organotin compound causes a dose dependent decrease in these amines in the whole brain of juvenile female rats. Maximum decrease was found to occur in the levels of DA. When estimated in different brain areas, hypothalamus and frontal cortex appeared to be the most affected, since levels of all the three amines were significantly lowered in these brain areas. A significant decrease in the contents of NA and 5-HT were also noted in cerebellum and pons-medulla. Additionally, the levels of NA and DA were also reduced in corpus striatum. A similar pattern for each amines was observed in the remaining brain areas in both groups (40 and 80 mg/kg) of DBTL exposed rats.

Certain organotin compounds resulted in the reduction of growth, food intake and instance of anemia. Unpalatability of diet, due to mixing of organotin compounds, has been suggested to be one of the factors for such effects. The present study also showed that feeding of DBTL to rats resulted in reduced body weight-gain, lethargic conditions, hindlimb weakness and swelling around the mouth area. Unpalatability of food can be ruled out in this study, since the animals received the organotin compound by oral intubation. Lower food intake due to sluggish conditions of rats or low absorption of nutrients from gastrointestinal tract may be responsible for decreased body weightgain in DBTL exposed rats. A generalized illness of animals after feeding of dialkyltin compounds have been reported earlier. Muscular weakness and paralysis has been observed in the animals exposed to organotin compounds, and similar observations have also been reported in humans.

Mortality index was highest in the juvenile and adult rats and comparatively lower in the weanlings. Rapid metabolism of DBTL and formation of toxic metabolities in the liver of juvenile rats may partly account for these effects, on the other hand an incomplete formation of biotransformation pathway and a poorly developed blood brain barrier may possibly explain the lower rates of mortality in weanling rats. Such a diversity in the rate of metabolism of organotins has already been reported in the literature which further supports our data. Further a higher degree of mortality in females at all ages indicates a sex related effect of DBTL, which could be chiefly due to harmonal influence. Variations in harmone levels or female sex harmones could affect the metabolism of DBTL, leading to a higher concentration of toxic metabolities.

Catecholamines and serotonin act as modulators of number of important behavioral functions, i.e. arousal, themoregulation sensory perception, emotional and aggressive behaviour. Alterations in the levels of these amines due to exposure of drugs such as amphetamine, apomorphine, or neurotoxic chemicals e.g. manganese, acrylamide, styrene, methylmethacrylate and organotins have been found to lead to disturbances in these functions. The present study showed that DBTL, like other organotin compounds, affected the levels of DA, NA and 5-HT and also the behavioral parameters.

Neurochemical analysis of regional brain biogenic amines in juvenile female rats depicted maximum alterations in hypothalamus and frontal cortex regions which registered a significant decrease in NA, DA and 5-HT levels at both 40 and 80 mg/kg doses, while corpus striatum, pons medulla and cerebellum showed a significant

decrease only in DA and 5-HT levels. Although there was no uniform pattern in regional changes in these amines, the magnitude of these alterations exhibited a dose dependent effect. This could be due to the variations in the chemobiodynamics of DBTL in discrete brain areas. More or less a similar pattern of changes in the levels of biogenic amines has been reported in rats exposed to acrylonitrile and dimethyltin. The cell bodies containing NA, DA and 5-HT are localized in distinct neuronal pathways, the fibres of which innervate and terminate in discrete brain parts. Our observations of significant reduction in brain biogenic amines in selected brain areas may partly account for the observed behavioral changes in DBTL treated rats.

Hypothalamus play a pivotal role in neuroendocrine regulation and the observed sex related effects may be explained to certain extent due to the alterations in three amines markedly in this brain region and cerebral cortex which controls motor function. Though in the present study assessment of brain biogenic amines in males of juvenile rats has not been carried out but the observed behavioral alterations indicate an impact of hypothalamic control on the neuronal correlates of behaviour i.e., motor activity and learning ability.

The role of DBTL in the neurobehavioral and biochemical alterations needs further confirmation with related biochemical parameters such as turn over rate, metabolic elimination and enzyme of the synthesis/degradation pathway of biogenic amines. However, the present study concludes an age, sex and dose related effect in the onset and severity of toxic lesions in DBTL intoxication.

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Dedicated to

My Lovable and
kind Hearted
Parents whose
love, affection,
devotion and
patience made
my efforts succeed

C E R T I F I C A T E

This is to certify that the work embodied in this thesis entitled **Chemical, Physical & Biological Factors in the Toxic Effects of Leachable Plastics Additives** has been carried out by Mr. Mohammad Shamshad Alam, under our supervision.

He has fulfilled the requirements for the Degree of Doctor of Philosophy in Chemistry of the Aligarh Muslim University, Aligarh regarding the nature and period of investigational work. The work included in this thesis is original unless stated otherwise, and has not been submitted for any other degree.

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A C K N O W L E D G E M E N T

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MOHAMMAD SHAMSHAD ALAM

M.Sc., M. Phil

CONTENTS

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ABBREVIATIONS

APL	-	Above than the permissible limit.
BIS	-	Bureau of Indian Standards [Old name Indian Standards Institution, (ISI)].
BPF	-	British Plastic Federation, U.K.
CAR	-	Conditioned Avoidance Response.
CNS	-	Central Nervous System.
CPM	-	Counts per minutes.
CPVC	-	Chlorinated polyvinyl chloride.
CS	-	Conditioned Stimulus.
DBTL	-	Dibutyltin Dilaurate.
DA	-	Dopamine
DEHP	-	Di(2-ethylhexyl) phthalate
DMF	-	Dimethyl formamide.
FDA	-	Food and Drug Administration, USA.
GM	-	Global Migration.
GR	-	Global Residue
gm	-	gram.
5-HT	-	5-Hydroxytryptamine.
ITI	-	Intertrial Interval.
ID	-	Internal Diameter
Lit.	-	Litre
MEHP	-	Mono(2-ethylhexyl)phthalate
min.	-	minute.
mg	-	miligram
NAPL	-	None above than the permissible limit.
NE	-	Norepinephrine or Noradrenaline
NF	-	National Formulary, USA.

ppm	-	parts per million.
Pharm.J.	-	Pharmacopoeia of Japan.
PVC	-	polyvinyl chloride.
PL	-	Permissible limit.
SE	-	Standard Error.
TMT	-	Trimethyltin
TET	-	Triethyltin
US	-	unconditioned stimulus.
UV	-	Ultraviolet.
VC	-	Vinyl Chloride.
ug	-	microgram.

PREFACE

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The rapid industrialization of the petrochemical industries and successful GREEN REVOLUTION have introduced a large number of pollutants into our environment. Among these plastics and chemical additives used during processing of plastics assumes a great significance. Today, plastics are being increasingly used in house hold, be it living room, kitchen or bathroom., food industry for packaging, storage and delivery of food., in hospital for renal dialysis, openheart surgery, tissue implants and storage, packaging and delivery of life saving fluids and drugs. They are also widely used in building materials, electronics and transport. Plastics are preferred over the other conventional materials like glass, wood and steel due to their light weight, rust less, mould resistance and wide range of colour acceptability. There has been a tremendous increase in the production and consumption of plastics during the last two decades. The total plastic production increased from 0.24 lakh tonnes in 1965 to 4.92 lakh tonnes in 1985 and is expected to be 24.4 lakh tonnes by the end of this century. The present indigenous capacity for different plastic raw materials is about 3 lakh tonnes per annum and per capita consumption is 0.58 kg in our country.

Plastics are basically organic polymer, constructed by chain like attachements of building blocks called monomers and some supplementary agents known as additives. In comparison to other industrial chemicals, plastics are comparatively new and not much is known about their toxicological effects. Rapidly increasing direct and indirect contact of human body with plastics have attracted the attention of health scientists all over the world on the possible health hazards of these polymers and the chemical additives used in their processing. Finished plastics are generally considered to be

safe, if they are used properly and manufactured by the chemicals recommended by National and International Regulatory Agencies. The unreacted monomers, copolymers, and chemical additives used in its processing are reported to leachout into the stored commodity under certain conditions and have been a cause of concern due to their toxicogenic potential. Migration of chemical additives from finished plastic articles into foodstuffs and biological fluids have become both a scientific and a regulatory concern.

The workers involved in the production of various chemical additives, handling and processing of plastics have been found to suffer from diseases like dermatitis allergic responses, bronchial asthma acroosteolysis with scleroderma, Raynauds phenomenon, hepatic and nervous disorders and even cancer. These diseases have been attributed to the monomers, and chemical additives used in the manufacture of plastics. Besides industrial workers, general population including pregnant mothers and children are also exposed to certain levels of the chemical additives for prolonged period of time due to their leaching from finished plastics and contamination of the food chain as a result of their use as fungicides. Migration of the chemical additives above than the permissible limit may be hazardous for the consumers of plastics on long term basis due to the toxic nature of some of the additives.

Regulatory agencies in different Countries have laid down guidelines for the safe use of plastics. In our country Bureau of Indian Standards (BIS) and Central Committee for Food Standards, Ministry of Health and Welfare made some guidelines for the testing of plastic materials for use in food and biomedical applications. In our country the materials stored in plastic pouches/containers in most cases are often subjected to high temperature and sunlight. In rural area and also in some of the urban area plastic

containers are used for the storage of food materials which has an acidic pH eg., pickles and are exposed to sunlight for curing. Usually fruit juices, vegetable oils, transfusion fluids and medicines are stored in plastic pouches/ containers for prolonged period than anticipated and are exposed to high temperature in summer season during delivery. The specifications laid down by BIS, for the safety evaluation of the plastic materials have not taken full account of such specific conditions, prevalent in our country. The leachability of the chemical additives could be modified by physico-chemical factors and may render the materials harmful.

Among the leachable components, plasticizers and stabilizers have been of a great concern. A significant amount of data on the toxicity of phthalate esters is available in literature. However, adequate information on the stabilizers particularly organotin stabilizer is not available. Since growing children may also be exposed to these stabilizer due to their leaching, it would be of interest to study the role of age and sex in the toxicity of organotin stabilizer.

In the present investigations, the candidate has studied in depth the effect of some physico-chemical factors such as sunlight, temperature, storage time, pH and chemical nature of the extracting media on the migration of chemical additives from plastic materials used for the storage, packaging and delivery of food, drinking water and blood bags. During the investigational period, safety evalution of 35 brands of plastic materials were also performed to generate knowledge/information about the quality of plastics available in the local market and their safe use. In order to assess the neurotoxic potential of Dibutyltin Dilaurate, a leachable stabilizer, its effects on biogenic amine levels, locomotor activity and learning ability have also been performed.

Attempts were also made to investigate the role of age and sex on the neurotoxic effects of DBTL since no information was available on this aspects of the widely used organotin stabilizer.

REVIEW OF LITERATURE

Plastics are basically organic polymer, constructed by chain like attachment of individual building blocks called monomers. They are defined in the Modern Plastics Encyclopedia (1982) as a "large and varied group of materials which consist of or contain as an essential ingredient, a substance of high molecular weight which while solid in the finished state, at some state in its manufacture is soft enough to be formed into various shapes, usually through the application, either singly or together, of heat and pressure.

The origin of plastic industry is intimately connected with the early growth of rubber industry. The milestones on the way to growth of rubber industry and indirectly that of plastic industry are the discovery of Hancock's rubber masticator (1820), the valcanization process (1833) and Schoenbeuin (1845) with the discovery of cellulose nitrate or celluloid. Some of the product achievments are the introduction of ebonite (1851), cellulose nitrate (1916) by Hyatt, Caesin (1919), alkid (1926), cellulose acetate (1927), polyvinylchloride (1927) and urea formaldehyde (1929) by Bakeland. The pioneer work of Carothers on nylon and polyster, the establishment of macromolecular concept by Standinger (Nobel prize, 1953), discovery of synthetic polybutadiene (1940), discovery of stereo regular polymers by Zieglernatta (Nobel prize, 1963), Flory's masterpiece treatment of polymerization kinetics and thermodynamics (Nobel prize, 1974) etc. are work to recall (1). The period after 1930 could be called as the materials age when a phenomenon growth of plastics took place and they started replacing, substituting and even competiting the conventional raw materials.

Application of plastics is rapidly increasing in our country. It is difficult to conceive a world without plastics which alongwith rubber have

revolutionised the world of materials. Ranging from day today home appliances and utensils, biomedical devices, industrial and agricultural products, automobiles upto space vehicles are been made from one or other kind of plastics. They are also being used as the building materials. The biomedical applications of plastics include their use as heart valves, vascular grafting material, intrauterine devices, catheters, disposable syringes, dialyzing sets, pouches for blood transfusions, dextrose, saline and other life saving fluids during surgery (2-5). The plastic used in biomedical devices are shown in Table -1.

Specific plastics are used in specific areas, some of which having direct or indirect human contact has been shown in Table-2.

Due to their light weight, mould resistance, thermostability, wide range of colour acceptability, slow mechanical damping, more resistance to corrosive environments, good electrical resistance, ease in the conversion of resin to the final products and consumption of comparatively small amount of energy, plastics are being preferred over the conventional raw materials. Infact, plastics have almost replaced the conventional raw materials like wood, steel and glass etc and have in the import substituted them in a big way in this country. The various products substituted by plastics are shown in Table-3.

The plastic industry in India has become a major industry. It constitutes about 7000 units with a fixed capital of Rs.170 crores. The consumption of raw materials is estimated to be around 4.50 lakh tonnes and the industry employs around 1,03,000 workers. In total it gives employment to around 40 lakhs people. The large and small scale industries constitute around 15,000 units with a capital investment of Rs.4,000 million. The per capital consumption of plastics in India is around 1.3 kg which is not significant in comparision to 55 kg in UK and 100 kg in USA (1).

According to Indian Plastic Institution (IPI), the total consumption of commodity plastics is expected to grow from a modest 492,000 metric tonnes (MT) in 1985 to 2,440,000 MT by the end of the century which is shown in Table 4 (6).

Total demand for enginering plastic materials are expected to rise from 34,650 MT in 1985 to 1,73,5000 MT by the year 2,000 which is shown in Table-5.

The finished plastic is a chemical compound which has repeating units of a monomer and several other chemicals to give its desired shape, colour and other properties. These chemicals are collectively referred as additives. The chemical additives are added to impart desired properties to the final item. There are nearly 2500 individual chemicals or mixtures utilized in various types of plastics and can be grouped in 14 major classes of additives. The purpose of these additives is briefly described below:

1. Plasticizers:

Plasticizers are mainly used in the thermoplastic resins and are added to impart desired flexibility, softness and processibility to the finished polymer. They may constitute in some cases more than 50% by weight of the finished product. Near about 450 plasticizers are commercially available. The commonly used plasticizers are Di(2-ethylhexyl) phthalate (DEHP), di-octyl phthalate (DOP), esters of the adipic acid and citric acid (7-8).

2. Foaming Agents:

Foaming agents are used in the cellular or foamed plastic products which are widely used as insulating and floatation materials. The main classes of foaming agents are: (a) azo compounds (b) sulfonyl hydrazides and (c) N-nitrosocompounds.

3. Initiators:

The polymerization of monomers is initiated by free radical mechanism. The commonly used polymerization initiators are (a) Protonic acids (H_2SO_4) (b) Lewis acids ($AlCl_3$) and (c) Organometallic compounds.

4. Antistatic Agents:

Antistatic agents in the manufacture of plastic material which is used in electronics such as flexible PVC, films and sheets. Antistatic agents are usually hygroscopic agents and attract and incorporate small amounts of moisture to the plastic surface. There are about 180 different type of chemicals used as antistatic agents.

5. Flame Retardants:

The flame retardants are added to high performance thermoplastic resins to make them suitable for application at high temperatures. The major chemical compounds used as flame retardants are: (a) Phosphate esters (b) Chlorine containing aliphatic, cycloaliphatic and aromatic compounds (c) Bromine containing aliphatic, cycloaliphatic, aromatic and ionic compounds (d) Chlorine, bromine and phosphorus compounds and (e) Inorganic compounds.

6. UV. Absorbers:

These are added to protect the plastics from deterioration by sunlight and fluorescent lightning. The most widely used U.V. absorbers are: Benzophenones, Benzotriazoles, Salicylates, Acrylates, Organonickel derivatives and amines.

7. Fillers and Reinforcements:

Fillers are used to reduce the cost of plastics. It induces less creep, greater rigidity, improved hardness and heat resistance. Commonly

used fillers are: (a) Silica products (b) Silicates (c) Glass (d) Calcium (e) Metallic oxides (f) Metal power (g) Carbon black (h) Cellulosic fibres and (i) Miscellaneous organic compounds.

8. Colorants (Dyes and pigments):

Dyes and pigments are used to impart desired colour to plastics. The colorants used are: (a) soluble dyes (b) organic dyes, pigments (c) Inorganic pigments (Titanium dioxide and iron oxide) and (d) some special compound.

9. Solvents:

Solvents are employed at a number of stages in the processing of some types of plastics. The major chemical classes of solvents used are: (a) alcohols (b) esters (c) glycols ethers (d) ketones (e) Nitroparaffins and (f) glycidyl ether.

10. Heat Stabilizers:

Heat stabilizers are used to prevent degradation of plastics by reacting with atmospheric oxygen. Thermal degradation occurs when plastics are exposed to higher temperatures or prolonged heating. The organotins (e.g. di-N-octyltin mercaptide, dilauryl and dibutyltin maleate), metal salts (e.g. inorganic salts of barium, cadmium zinc and lead, usually as phosphates, carboxylates and phenates), epoxides and pentavalent phosphorus compounds are the major chemical stabilizers. The organotin stabilizers are used in the range of 0.3 to 1.5% of resin for chlorinated polyvinyl chloride pipes (PVC) and fittings (9).

Toxicity of the various leachable chemical additives such as phthalate esters, heavy metals and monomers such as styrene, acrylonitrile

and vinylchloride are well known. However very little is known about the toxicity produced by organotin compounds which are widely used as heat stabilizers in the plastic industries. Therefore, the candidate has studied in depth the toxic effect of dibutyltin dilaurate, a leachable plastic stabilizer; using albino rats as the experimental model.

ORGANOTIN COMPOUNDS

Organic-tins are defined as compounds with a covalent Sn-C bond and may be represented in the general form as:

$$Rn\text{-}Sn\text{-}X_{4-n}$$

Where,

Rn= one to four alkyl or aryl groups.

Sn= the central tin atom having a covalence of four.

X = a singly charged anion or anionic organic group.

n = 1,2,3,4.

The inorganic nature of the tin atom is altered by the carbon-tin covalent bonds due to which the molecule behaves in a manner similar to substituted hydrocarbons.

INDUSTRIAL & AGRICULTURAL APPLICATIONS OF ORGANOTINS:

The organotin compounds have wide range of application ranging from industry to agriculture. Their applications are described below (10):

A - HEAT STABLILIZERS:

Organotin compounds are widely used as heat stabilizers to prevent the thermal degradation of chlorinated compounds such as certain types of transformer oils, polyvinyl chloride, Poly (vinylidene chloride), chlorinated

rubbers, parafins and modified plastic. These are also used to stabilize other nonhalogenated compounds such as some lubricating oils, hydrogen peroxides, cellulose acetate, polyamides, polycarbonates, polyethylene and polypropylene. There are approximately 1000 patents for organotin stabilizer formulations, but only few are of commercial values which are mentioned below:

1.	Dibutyltin Dilaurate	:	$(C_4H_9)_2$ Sn (00 $CC_{11}H_{23})_2$.
2.	Dibutyltin Maleate	:	$(C_4H_9)_2$ Sn(OOCCH = CHCOO)$_n$.
3.	Dibutyltin Di-(monobutylmaleate)	:	$(C_4H_9)_2$ Sn (OOCCH = CHCOOC_4H_9).
4.	Dibutyltin Mercaptopropionate	:	$(C_6H_9)_2$ Sn (SCH_2 CH_2 COO)$_n$.
5.	Di-n-Octyltin Maleate	:	$(n\text{-}C_8H_{17})_2$ Sn (OOCCH = CHCOO-)$_n$.
6.	Di-n-Octyltin thioglycolate	:	$(n\text{-}C_8H_{17})_2$ Sn (SCH_2 COO)$_n$.
7.	Di-n-Octyltin bis(2-ethylhexyl maleate	:	$(n\text{-}C_8H_{17})_2$ Sn (OOC.CH=COO CH_2-CH (C_2H_5)-(CH_2)-CH_3.

Dibutyltin dilaurate are generally used in rigid PVC products such as pipes, bottles, insulation etc while dioctyl derivatives are commonly used in PVC formulations which come in contact with food.

B. CATALYTIC AGENTS:

Urethane Catalysts : Organotin compounds are widely used as catalysts in the production of polyurethane foams and allow the foam to be made directly from hexamethylene diisocyanate and 1,4-butanediol.

The most commonly used organotin catalysts are: dibutyltin acetate, dibutyltin dilaurate, dibutyltin dichloride, dibutyltin dilaurylmercaptide, and

dimethyltin dichloride. Several stannous compounds, particularly stannous octoate has been also used very successfully in flexible urethane foam applications.

Dibutyltin dioctoate and dibutyltin dilaurate are commonly used to catalyze the room temperature curing of silicone rubbers used in making dental impressions and encapsulating electronic parts.

Organotin compounds have distinct advantages as catalysts for esterification reactions. They have(a) high catalytic efficiency, (b) low tendency to eliminate water from secondary alcohols to form olefins., (c) ability to produce colourless esters., (d) absence of acidic or basic residues in the esters., (e) ability to impart heat stabilization to condensation type polymers (i.e. polyesters); (f) ability to improve physical and electrical properties of the product.

BIOCIDAL COMPOUNDS:

One of the most important application of the organotin compounds have been found to be as a preservatives for wood, textiles, cordage fibers, paper, leather, electrical and electronic equipment and glass. Most biologically active organotin compounds are trialkyl or triaryltin compounds. Bis (tributyltin) oxide (TBTO) gives antibacterial, antifungal, and mothproofing properties to treated fabrics. TBTO has been found to be an extremely effective bactericidal agent in hospitals for staphylococcus aureus. TBTO has also been used to prevent odours in garbage pails, control athletes foot, control molds in bathrooms, control mildew on leather goods, textiles, and plastics and mothproof stored garments.

Industrial applications of organotin biocides include their use for slime control in paper pulp mills and cooling towers. Dibutyltin dilaurates

are effective for the control of Raillietina cesticillus in chickens, and the control of other poultry tapeworms. Dialkyltin compounds has also been used in the control of other parasitic diseases of poultry, sheep and swine. Tibutyltin chloride is an effective rodent repellent. Triphenyltin acetate and triphenyltin chloride are known to be an effective molluscicides for the control of snails which serves as vectors for schistosome infections in man. Tributyl- and isopropyltin compounds are effective fungicides.

Due to the expansion of technical applications, the annual world production of organotin compounds has grown from 500 tonnes in 1950 to 65 millions pounds in 1982 and it has been estimated that the demand of these compounds will be about 115 million pounds by 1990 (11). The consumption of organotin compounds for PVC heat stabilization is 67%., catalytic applications is about 8% and biocidal applications accounted for 20% by weight of all organotins.. The distribution of organotin compounds in various areas have been illustrated in the Figure-1.

The worldwide consumption of organotin compounds from 1977 to 1982 and the projected demand forecast from 1985 to 1990 has been shown in Table-6.

POSSIBLE SITE OF EXPOSURE TO ORGANOTIN COMPOUNDS:

The exposure of the industrial workers to the organotin compounds occur through dermal contact and injection. The quantity of the abundance varies from industry to industry and the mode and experiments of the application of organotin compounds. As a result of their widespread distribution and the contamination of the water and soil, the general population is also exposed to the organotin compounds. The general population is also exposed to organotin compounds due to their use in biomedical and food packaging

devices as heat stabilizers in PVC plastics by leaching process. Possibility of the exposure of organotin compounds to agricultural workers also exist as a result of their use as biocidal compounds. The possible way of environmental exposure of organotin compounds to the people of different areas has been represented in Figure-2. (13-14)

ENVIRONMENTAL FATE

Limited studies on the distribution of the organotin compounds in the environment have been undertaken so far. Approximately 10 lakh pounds of organotin compounds (0.4% of total production) are believed to be released annually in the environment by the manufactures. The specific amount of inorganic or organictins present in the environment have not been studied. Organotin compounds of various chain length have been detected in the environment, contaminated water, fishes and in the plants. The levels of organotin compounds in dried plants ranged from 1 to 10 ppm and inorganictin compounds in the same plant ranged from 20 to 200 ppm. Level of organotin compounds in fish meat and fish liver was found to be 1 ppm (11).

There is a possibility of toxic metal cycling in the environment and the tin is reported to crossover with mercury in the environment (15-17). A bimetallic mercury-tin (Sn-Hg) "Crossover" scheme of toxic metal cycling has been shown in Figure-3.

CHEMICAL AND PHYSICAL PROPERTIES OF ORGANOTIN COMPOUNDS WITH SPECIAL REFERENCE TO DIBUTYLTIN DILAURATE (DBTL):

The structure of dibutyltin dilaurate has been depicted in the Figure-4.

1. Molecular Formula	:	(C_4H_9) Sn $(OOCC_{11}H_{23})_2$
2. Molecular weight	:	631.55
3. Tin Content (%)	:	18.8
4. Appearance	:	Liquid or solid at low melting point
5. Bioling point (0°C/mm Hg)	:	400/10 mm Hg
6. Specific gravity at 25°C	:	1.05
7. Melting Point, °C	:	27
8. Solubility	:	8 to 50 ppm soluble in water and readily soluble in organic solvent e.g. Chloroform or benzene.

Chemical reaction of the tin-carbon bond include acidic and basic hydrolysis (or solvolysis in general), oxidation, photooxidation and reduction. The tin-carbon bond is quite stable and resistant to oxidation below 200°C. However, in solution the bond may be polarized by attacking molecular or ionic species and may readily undergo cleavage with loss of one or more organic substitutes attached to the tin atom (12).

Kinetic studies of organotin decomposition in solution have established that solvolysis, including hydrolysis, is second order, i.e., first order with respect to the organotin molecule and first order with respect to the attacking species. Many kinetic studies have been performed with large excess of attacking species which yields pseudo first order kinetics in order to simplify data interpretations.

Cleavage of organotin in solution in pH dependent and in general, requires conditions of high acidity (PH $<$ 4) or high alkalinity (PH $>$ 12).

For intermediate acidity, values pH 4 to 12, reaction rates are thought to be very slow.

Organotin which have one or more unsaturated substituents are more easily cleaved than saturated groups of the same number of carbon atoms (18). A second alkyl group may be attached before the first group has been completely removed yielding a complex set of products. Organotin may be reduced in solution by strong reductants e.g. sodium borohydride, to form series of substituted stannanes or stannane itself, Sn H. (19-20).

Migration of organotin stabilizers from plastics:

Organotin compounds are reported to migrate in varying quantities from PVC bottles and other containers during storage. Large amounts of organotin stabilizers have been detected in tap water, urine and olive oil stored in flexible PVC as compared to rigid polymer (21). They have also been detected in whisky, beer, cherry soda, apple juice, vegetable oils, peanutoil, sunflower oil, tomato juice and mineral water stored in plastic bottles stabilized with organotin compounds (22-25). The ability of biological fluids to extract organotin heat stabilizers from plasticized PVC medical devices has also been well documented (26-30). The concentrations of organotin compounds in various solutions, liquid foods and fruit juices was about 2 ppm. However, the concentration of organotin in foods should not be more than 1 ppm according to the recommendations of Food and Drug Administration (10). The National Sanitation Foundation has set up a limit of 0.05 ppm extractable organotins from plastics as total tin (11), and West German Govt. has set up the daily tolerance level, 0.0065 mg/kg for humans.

Metabolism:

The absorption of linear alkyltin compounds has not been studied well. However, it is known that triethyltin & trimethyltin compounds are well absorbed from gastrointestinal tract, due to their solubility, while other alkyl, aryl and inorganic tin compounds, being rather insoluble, are poorly absorbed (31-32). Some investigators using C^{14}-di-n-octyltin have detected only small amount of radioactivity in liver, kidney, spleen, brain, muscles, heart, lung, serum, adrenal, pituitary and thymus, while the rest were excreted through faeces and urine (33). Partial conversion of diethyltin to ethyltin has also been shown using C^{14}-labelled ethyltin compounds (34). Monodiethylation of tetraethyltin compounds in rat's liver has been reported (35). Absorption of small amount of organotins through the gastrointestinal tract, gets mainly distributed in liver, kidney, from where it disappeared within few weeks has been reported. The metabolism of tetraalkyltin compounds to trialkyltin compounds have been shown to proceed rapidly in the animals (35). The major site of metabolism of organotin compounds is liver. The alpha, beta, gamma and delta hydroxylation of triethyl, tripropyl, tributyl and trihexyltin compounds have been reported and is carried out by the hepatic cytochrome p-450 dependent monooxygenase system (36-38). It has been reported that monoethyltin given orally to the rat appeared only in faeces and was not detectable in urine, suggesting poor absorption; on the otherhand, after i.p. injection of the same compound appeared only in urine (34).

TOXICITY OF ORGANOTIN COMPOUNDS:

Many of the organotin compounds are reported to be toxic. The manifestations of the toxicity are dependent upon the organic constituents of the tin compounds, the mode of [illegible]

studied, as shown in Table-7. The acute LD_{50} of some of the important organotin compounds have been summarized in Table-8.

The toxicity of the organotin compounds decreases with the increase in n-alkyl chain lengths within triallkyltin series. The highest toxicity has been observed for triethyl tin compounds. Further increase in normal alkyl chain length produces a sharp drop in biological activity and tri-n-octyltin compounds become almost non-toxic to all living species. Dialkyltin compounds show a similar trend of decreasing toxicity with increasing length of the alkylchain. However, the symptoms of poisoning produced by dialkyltin compounds are entirely different from those produced by trialkyltin compounds. The monoorganictin compounds do not exhibit any important toxic action in mammals and show the familiar pattern of decreasing toxicity with increasing chain length (39). It has been shown that the commercially important thermal stabilizers and catalysts are less toxic than the trialkyl or triethyltin compounds. With the increase in size and stability of organic ligand, toxicity of these compounds were found to be reduced (10).

The hazards associated with the use of organotin (TET) were unmasked by an episode of intoxication in 1954 in France, involving over 200 cases, 100 of which were fatal. Predominant symptoms and signs of toxicity were severe headache, nausia, gastric pain, dryness of mouth, visual and psychological disturbances, shortness of breadth and sometimes loss of conciousness, hepatomegaly and elevated levels of liver transaminases activity (10 & 45). Occupational exposures have accounted for two reported episodes of TMT poisoning. The first episode involved two chemists who were involved in the synthesis of dimethyltin and were inadvertently exposed to vapours of dimethyl-and trimethyltin

chloride for approximately 3 months (46). The second episode involved twenty two workers who were exposed to both dimethyl and trimethyltin dichloride and methylbromide due to the malfunctioning of the ventilation system (47). Vapours of triethyltin acetate produced headache., general weakness, nausia and diarrhoea (48-49).

The acute toxicity resulting from organotin exposure to animals has been reviewed by Stoner et al (50). Triethyltin (TET) was the most potent, although other alkyltins produced similar sign of poisoning. The acute toxicity of triethyltin (TET) and trimethyltin (TMT) has been described as being qualitatively similar. Trembling, irritation, twitching, loss in body weight and progressive paralysis (51-52) has been reported. weight loss and impaired growth were observed in rats fed upto 200 ppm triphenyltin acetate diet, Guinea pigs were more sensitive and impaired growth occurred at 1 ppm of triphenyltin acetate (53).

BIOCHEMICAL BASIS OF ORGANOTIN TOXICITY:

The biochemical basis of organotin toxicity has been studied extensively (20). The interference in the functioning of mitochondria by triorganotin compounds was observed and are of three kinds:

1. an energy dependent chloride hydroxyl exchange, where the most potent compounds are small molecules distributed fairly equally between lipid and water (i.e. TMT).
2. an energy conservation mechanism where the most potent compounds are/lipophilic and of higher molecular weight (i.e. triphenyltin) and,
3. a generalised interference with mitochondrial membrane. The first two effects were related to be binding of the tin compounds rather selectivity to a few sites on certain proteins (20).

One type of binding depends on a balance between lipid and water solubility at physiological pH, the other to a potential for 5-coordinate binding by tin.

EFFECT ON BLOOD:

The effects of leachable additives of plastics on human serum protein and antibodies has been studied Bis-dibutyltin monolaurylmaleate cause no change in blood grouping antiserum, but produced red blood corpuscles agglutination. Dibutyltin di-isooctylmaleate was found to be destructive to antibodies and affected the blood reagents ability to selectively agglutinate human blood cells. This compound also caused human cells to lyse within 24 hours (54).

EFFECT ON REPRODUCTION:

Reproductive system is also affected by organotin compounds. A reduced fertility in early days of experiment in male rats., which improved later has been reported (55). An increased number of resorption after octyl tin-s-s'bis(iso-octyl mercaptoacetate) administration to rats has also been shown (56).

CELL CULTURE STUDIES:

It has been reported that organotin compounds cause cell necrosis in both mouse fibroblast and chick embryo tissue culture tests (57). The observation that the plasticized plastic produced cell necrosis while unplasticized caused very little or no cell destruction (10), implied that the presence of plasticizer helps in leaching of stabilizers.

Effect on Liver:

Under in vitro conditions uncoupling of oxidative phosphorylation of rat liver homogenate and mitochondria has been reported following

exposure to triethyl tin compounds (58). Mitochondrial swelling and inhibition of both magnesium and 2,4-dinitrophenol activated adenosine triphosphatase of isolated rat liver mitochondria has been observed. Exposure to Dibutyltin Dilaturate in rat liver resulted in a consistent decrease in the activity of aniline hydroxylase, benzo(a)pyrene hydroxylase, aminopyrene-n-demethylase, benzphetamine-n-demethylase and content of cytochrome P-450 (59). In addition to this, an increase in the free -SH content and decrease in the bound-SH content was also noticed (59).

Neuropathology:

Neuropathological effects of TMT and TET have been well documented (60-76). Both the compounds have been found to damage the developing CNS. However, they have different cellular targets. TMT is a neurotoxin which damages areas of the limbic system, cerebral cortex, and brain stem (77-79). TET is a myelinotoxin which causes massive myelinic edema. Exposure to TET during the neonatal period has been reported to produce neuronal death as a result of elevated intracranial pressure (70-76). The neuropathological effect of TMT chloride on neonatal rat hippocampus resulted in the development of pathological lesions in the developing hippocampus and was found to be an age depedent and neonates were found to the most vulnerable. TMT exposed animals appeared to be significantly stunted in growth. Brains of TMT exposed animals were also markedly smaller than those of agematched controls. Extensive neuronal edema, accumulation of lysosomes and myelinoid membranous bodies and cellular necrosis were observed as early as 4 days after exposure.

NEUROBEHAVIORAL TOXICITY:

Organotin compounds have been reported to produce neurobehavioral disorders in human beings and animals. Their toxic effects

have been known since 19th century (81-81) and invariably has been observed in workers involved in the synthesis of specific organotins. It has been reported that triethyltin inhibits selectively the oxidation of glucose in rat brain (70,82). A decrease in the incorporation of ^{14}C-glucose into glutamate, glutamine alpha-aminobutyrate and aspartate was also observed in the treated animals. The experimental data indicated that the triethyltin was able to decrease the rate of pyruvate oxidation (35). A decrease in the levels of noradrenaline and 5-hydroxytryptamine was also observed in the brain of the rats exposed to organotins for few hours (83). The central neurotransmitter effects to TET and TMT in rats by measuring the concentration of dopamine, norepinephrine, homovanillic acid, dihydroxyphenylacetic acid, γ-aminobutyric acid (GABA), acetylcholine and choline in different brain areas showed that TET has no effect on any of the parameters measured, whereas TMT significantly decreased GABA and dopamine levels, but the decrease was found only in hippocampus and striatum respectively (79,84). Dose dependent alterations in the uptake of endogenous glutamic acid and GABA have also been reported in synaptosome isolated from matured rat hippocampus (84).

Effect of TET bromide on mitochondrial membrane permeability has also been investigated (85-88). The following conclusions have been drawn by this study:

1. TET may have a direct action on the terminal membrane which results in interference of the release mechanism for Ach.
2. TET may inhibit the production of the Ach precursors, acetyl COA, by interfering with mitochondrial function.

3. TET may interfere with intraterminal Ca management by altering Ca uptake by mitochondria and, thus, interfere with the controlled release of Ach.

4. TET may directly effect anion flux in muscle membrane which results in a decrease Resting Membrane Potential (RMP), and may also explain the increased threshold of stimulation and changes in baseline tension during stimulation.

5. TET may have indirect effect on the sodium pump by inhibiting the mitochondrial production of ATP and thus in parts may contribute to the decrease in muscle RMP.

The behavioral effects of triethyltin (TET) and Trimethyltin (TMT) exposure to human has been summarized in Table -9.

Behavioral Effect of TET:

The behavioral changes caused by triethyltin (TET) in mammals has been shown in Table 10. TET produces primarily neuromotor impairment which is reversible. Following subacute administration of 5 or 10 ppm TET in the drinking water, decrease in locomotor activity within 2 weeks and after removal of TET from the drinking water, the motor activity of TET exposed animals had returned to control levels (89). The startle response to both acoustic and tactile (air-puff) stimuli (89-90) was reduced during exposure to TET and the effect were found to be reversible on the termination of exposure. Reduction in the operant response rates on both fixed interval and fixed ratio schedule of reinforcement as a result of exposure of TET to rats has also been found (91). The conditioned flavour aversions have been consistently reported to reduce following acute and repeated exposures to TET. Decrease in grip strength, motor activity, and startle response amplitudes has also been noticed in rats

exposed to TET (92-94). These effects were found to be usually reversible, 2-4 weeks after the termination of exposure.

Behavioral Effects of TMT:

The behavioral changes caused by the exposure of TMT has been summarized in Table 11. Rats exposed to 7 mg/kg TMT were found to be more active than controls (95), the animals became progressively hyperactive from day 4 to 16 day after dosing. This time course parallels the development of limbic system damage, which first appeared 2 days after dosing and becomes maximal within 3 weeks (60). Animals exposed to TMT were less susceptible in selecting arms of the maze, and required more arm enteries to obtain all reinforces; this deficit in performance existed for at least 70 days after dosing (96). Effects of TMT exposure on performance in a Hebb-Williams maze have also been evaluated. Animals receiving 7 mg/kg TMT made more errors than controls and also made more perservative responses, repeatedly entering blind alleys which did not lead to reinforcement (97). Impair retention of a passive avoidance task and facilitation in active avoidance performance indicated that TMT exposure impairs acquisition and/or performance of tasks involving learning and memory. TMT exposure has also been found to affect the schedule controlled performance. Response rates on a schedule with increasing fixed-ratio requirement were found to be increased by TMT. On the other hand a decrease in the rate of responding in TMT exposed mice have also been reported (98).

Health Hazard of Plastics:

Finished plastics are genrally considered to be safe if they are used for the purposes for which they have been designed. However,

the workers involved in the production of various chemical additives such as plasticizers, stabilizers, colorants, pigments and processing of plastics have been found to suffer from diseases like dermatitis allergic responses, bronchial asthma , acroosteolysis with scleroderma, Raynauds Phenomenon, hepatic and nervous disorders and even cancer (104). These diseases have been attributed to the monomers and additives used in the manufacture of plastics. Besides industrial workers the general population including pregnant mothers and children are also exposed to the chemical components of plastics i.e. plasticizers stabilizers, heavy metals, unreacted monomers etc., due to their leaching from finished plastics into the stored commodity and contamination of the food chain as a result of their use as fungicides. Though no reports are available regarding the health hazards caused by the direct use of plastics in India or abroad., however on the long term basis the various decreases related to the compounds may develop to the consumer due to exposure of some of the injurious leachable chemicals. Migration of phthalate, a plasticizer from PVC bags into blood, milk and other life saving fluids have been reported (105-107). It has been shown that Di-(2-ethylhexyl) phthalate (DEHP) a widely used plasticizer is hepatotoxic, mutagenic and even carciongenic (108-110). Studies conducted in our laboratory has shown leaching of heavy metals such as Pb, Cd, Mn, Cr, Cu & Zn etc., in concentrations above than the permissible limits and concentrations of Pb, Cr, Cu & Cd were more than daily intake values in drinking water for human beings from plastic materials used in biomedical devices and food packaging (111-112). Experimental studies have shown that heavy metals are injurious to health and produce a large number of disorders. Manganese (Mn) and Lead (Pb) are reported to produce neurotoxicity while cadmium (Cd) is a nephrotoxic element (113-115). Chromium

(Cr), Copper (Cu) and Zinc (Zn) have been reported to produce alterations in the reproductive organs and skin diseases (116-118). The candidate has shown leaching of some of the u.v. abosorbing materials in concentration above than the permissible limits from some of the plastic materials studied (119). The migration of u.v. absorbing materials (e.g. derivatives of benzophenone, benzotriazole, salicylates, acrylates, organonickels and amines) above than the permissible limits may also be hazardous as some of these are reported to be toxic (120). Experimental studies have shown that acrylates, benzophenones, benzotriazoles and amines produced skin irritations (121-125). Due to excess exposure of benzotriazole the animals developed mutagenic effect (121). Patients exhibited asterixis, abnormal mental state, tinnitus and deafness as a result of salicylate ingestion (122-124 and 126). Pulmonary edema has also been reported by many investigators in the patients due to excess salicylates ingestions (127-130). The derivatives of benzophenone have been reported to induce pawedema in rats and immuno-suppressive activity and anti-inflammatory effects in human (125). The finished plastic often contain unreacted (basic) monomers such as vinylchloride, acrylonitrile, methylmethacrylate and styrene. These monomers have also been reported to migrate into stored commodity from plastics (131-138). Vinylchloride (VC) has been reported to cause terminal cancer in the workers of the PVC industry (134). It has also been shown that the workers engaged in PVC production and polymerization were more susceptible to the enormous risk of liver malignancies and increased risk of brain and CNS tumours and perhaps also to malignancies of the lymphatic and hematopoietic system (139). Acrylonitrile present in the acrylic and modacrylic fibres, resins and rubbers is reported to cause irritation and allergic dermatitis, nausea, vomiting, headache, immunodepression symptoms of gastritis, colitis

and belepharoconjunctivities to humans (140-147). Styrene present in polymers, copolymers and reinforced plastics is reported to cause hepatomegaly, splenomegaly, leucopenia, lymphocytosis, skin atrophy, neurogenic muscular atrophy, anxiety reaction, headache, sleepiness and peripheral neuropathy in humans (148-151). Methylmehtacrylates has been shown to leach out from dentures made of methylmethacrylte cement. The patients wearing such dentures have been reported to suffer from cardiac arrest and hypotension (152).

Effects of Plastic implants on tissue:

(a) Short Term Contact:

A plastic device placed in contact with the tissue, may release a constituent causing a local irritation response which may vary from a mild inflammatory response to a highly corrosive action. Certain type of plastics such as nylon epoxypolymers and polyurethanes have been shown to be associated with the tissue responses.

Plasticizers, used to impart flexibility to the PVC polymer enhance the diffusion of organotin compounds from the materials. Such release leads to a tissue response. Table 12 shows the effect of organotin compounds in the presence of a plasticizer. As evident from the table, the organotin compounds in absence of the plasticizer produced a tissue reaction detectable for only a week, while in presence of the plasticizer a prolonged tissue reaction was observed (152-153).

(b) Long Term Contact:

Depending on the specific material and the site of implant, it is possible that materials implanted for long periods of time in animals and humans will degrade, releasing polymer fragments to the body which may elicit one or more biologic responses.

It has been demonstrated in rodents that all types of materials having contact with tissue for longer than six to none months leads to tumour production at the implantation sites. The kidneys of rat wrapped in cellophane, were found to bear tumours after a long term contact (154). It has also been demonstrated that the size and form of implant, surface, hardness, thickness and length of implants influence the tumorigenesis in experimental animals (155-161).

2. Allergic Response:

Acrylic denture materials are reported to produce responses in certain patients which are due to the monomer or one of the additives present in the completed denture (162). The presence of sensitizing agents in these materials could lead to allergic responses in some patients who are hypersensitive to the offending agent.

3. Systemic Toxicity:

The migration of constituents from the plastics or elastomers to the tissues may result in the absorption of that compound. If the compound is absorbed in sufficient concentration, it may lead to systemic toxicity. It has been reported that when the circulating blood comes into contact with the polymeric materials, it results in thrombocis formation. The blood clotting may be attributed to surface charge, Zeta potential, surface forces of the unspecific nature, surface structure, absorption of certain types of proteins from circulating blood and the rate of flow of blood (163).

The plastics for biomedical applications must be sterilized. Since the plastic materials cannot withstand autoclaving, other methods of sterilization are employed. Ethylene oxide or a combination of ethylene

oxide with inert gases have been used for the sterilization of the plastics. Residual ethylene oxide in plastics has been reported to lead to some health hazards.

Plastic Blood Problem:

Thrombus formation has been observed as a result of contact of circulating blood with the polymeric materials. Factors causing this blood clot have been attributed to surface charge, zeta potential, surface forces of unspecific nature, surface structure, absorption of certain types of proteins from circulating blood, and the rate of flow of blood (164). The blood-plastic clotting problem, however, has been in certain instances circumvented by treating the material with graphite, followed by benzalkonium chloride and finally by heparin (165). During recent years there have been approach to build into the polymer chain functional groups that will bind heparin. Newer experimental materials are being conceived in which the material has a "heparin" type functional group, which will solve the blood cloting problem.

Toxicity Due to Treatment of Materials:

The possibility of the potential hazards due to treatment of materials for a specific reason cannot be ruled out. In medical and dental applications it is necessary to have the specific devices that will have contact with tissue, be sterile, and be generally pyrogen free. The way to accumplish this could have been by the use of an autoclave. Unfortunately, most of the plastic products cannot with stand autoclaving, and thus other methods of sterilization such as by the use of ethylene oxide, a combination of ethylene oxide with inert carrier gas, quarternary ammonium salt (benzalkonium chloride) and coating of silicone liquids have been in practice.

However, toxic responses such as hemolysis in clinical and experimental studies have been observed due to ethylene oxide residues in plastics (166-169). The chemical responsible for hemolysis has been reported to be the ethylene chlorohydrin, as a result of sterilization of PVC plastic with ethylene oxide (170).

Adequate information about the effects of combined exposure of ethylene oxide and ethylene chlorohydrin to animals are not available in the literature. It has been anticipated that presence of benzalkonium chloride in sufficient concentrations can produce irritations in certain tissues. However, in most instances, these quarternary compounds were found to be in concentrations below than that which would produce an irritant response, and thus the anticipated problem has not been noticed. Ebolism and death has been reported due to release of silicone with the flow of blood through plastic tubings coated with silicone liquids (171). Accidents of this type are very much likely to happen when innovations are made for the purpose of improving a material without recognizing introduction of a more serious consequence.

Table - 1: Plastics being used in medical*

Class	Device or Item	Examples
A.	Permanent Implants	Heart values, various vascular grafts, Orthopedic implants, other artifical organs etc.
B.	Implants having contact with mucosal tissue	Artificial eyes, contact lens, dentures, Intrauterine devices, certain types of catheters.
C.	Corrective, protective and supportative devices	Splinters, Braces, Films and Protective clothes etc.
D.	Collective and administration devices	Blood transfusion sets, various types of catheters, Dialyzing units, hypodermic devices and similar injection devices etc.
E.	Storage devices	Containers, Bags for blood, Blood products, Drug products, Nutritional products and Diagnostic agents, etc.

* From Autian, J. : Development of standards for plastics to be used in pharmacy and medicine. J. Dent. Res., 45: 1668, 1966. Copyright by the American Dental Association. Reprinted by permission.

Table - 2: Major Applications of Polymers and Plastics having direct or indirect human contact.

Polymer	Significant property	Applications
ABS	Outstanding impact length, mechanical strength over wide temperature range, excellent colour and appearance, combines many of the best properties of acrylic rubber and styrene polymers.	Pipe and fittings, refrigerator parts, football, helmet, telephone boxes, impellers, automobile-decorative parts etc.
Chlorinated polyester	High thermal stability, high corrosion resistance, excellent insulation, good impact strength, high dimensional stability, low moisture absorption.	Pump parts, pipes, sheet, corrosion resistant coatings, chemical processing industries etc.
Epoxides	Tough, flexible, good adhesion, chemical resistance to a degree unattainable by other materials, electrical insulation.	Pipelinings, printed circuits, electronic devices, paints, fibre reinforced articles etc.
PVDF	Heat resistant, toughness, low coefficient friction to distortion and creep	Bakery trays, food processing equipment etc.
Olefine copolymers (EVA, EEA)	Long term flexibility, toughness, clarity, easy processing.	Automotive application, tubings wire and cable insulation, syringes, squeeze bulbs, toys, film sheetings, closers, automobile parts, packagings, industrial appliances and house wares etc.
Polypropylene	Stiffer, more heat resistant, withstand boiling water, carry high load, withstand fatigue, light weight, tough and brittle (low temperature).	Valves, packaging films, bottles, wire insulation, pipes & fittings, houseware, toys, washing machine tops, automotive applications, luggage etc.
Polystyrene	Transparent, hard, high strength, high refractive index, brittle, high dimensional stability, good water and chemical resistance.	Packaging, radio, housing, battery casings, kitchen appliances, food containers, toys, wall tiles etc.

Polyurethane	Resistant to abrasion, resistant to solvents, good flexibility, good impact resistant, versatile.	Foam products, cushioning mattresses, insulated clothings, toys packing materials, light weight structural parts etc.
Poly (Vinyl Chloride)	Hard, horny and brittle, but plasticized PVC offers a broad spectrum of properties.	Raincoats, pipes, tiles, phonograph records, luggage, toys, draperies, upholstery, wire insulation, foam work gloves etc.
Polybutylene	Similar to PE and PP more crystalline, high strength at low stiffness and resistant to deformation.	Pipe and Pipe fittings, films, etc.
Poly (Phenylene oxide)	Excellent electrical resistance, chemical resistance, mouldable, stable -275 to 375°C	Hot water values, replacement for glass and stainless steel in medical utensils, plumbing fixtures, replacement of stainless steel in missile, computer parts etc.
Polyvinylidine-chloride).	Weak transparency, pliable plastic.	Upholstery, fabrics, screenings, pipes, tubings etc.
Polyallomers	Rigidity, high impact strength, light weight, superior, to PE in processability, and stress crack resistance.	Food and heat sterilizable containers etc.
Styrene-acrylonitrile copolymer	Better electrical resistance, surface hardness, scratch resistance.	Lenses, decorative panels, cups, tumblers, typewriter keys, refrigerator parts etc.
Styrene-butadience	High impact resistance	House wares, light fittings, refrigerator components, radio and TV cabinets, packagings etc.
Urea-formaldehyde	Water white clarity, hard and tough, good light diffusivity, resistance to oil, grease, organic solvents but attacked by conc. acids and alkalies.	Coatings, kitchen were table ware, radio cabinet, insulation foam, button, bottles, cups, toilet seats, etc.

Table - 3: The raw material substituted by plastics

Plastic Products	Raw Materials substituted
PVC and HDPE Pipes	Zinc required for galvanised iron pipes.
HDPE buckets and bins	Zinc required for galvanised iron buckets.
PVC and XLPE cables	Lead, oil and paper required for PVC cables.
Polypropylene ropes	Manila fibres required for ropes.
Bi-axially oriented polypropylene film.	Alpha Cellulose required for making films.
HDPE blow moulded containers	Tin required for plated containers, cold roller steel required for mild containers.
Polypropylene refills for ball pens.	Copper required for brass refilles.
LDPE roto moulded containers	Stainless steel required for pickling bath.

Table - 4: Expected consumption of plastics (1980-2000).

Types of Polymers	1980	1985	1990	1995	2000
LDPE/LLDPE	91,000	146,000	255,000	410,000	650,000
HDPE	60,000	115,000	205,000	378,000	571,000
PVC	108,000	165,000	270,000	432,000	704,000
PP	19,500	40,000	85,000	186,000	384,000
PS	12,500	26,000	51,000	81,000	131,000
Total	290,500	492,000	866,000	1,487,000	2,440,000

Table - 5: Demand forecast of engineering plastics - 1980-2000

(In Million Tonnes)

Type of Polymers	Year				
	1980	1985	1990	1995	2000
ABS	1,200	3,300	15,500	27,300	40,000
Nylon	795	2,350	7,000	12,050	17,500
Polycarbonates	NA	1,000	3,000	6,000	10,000
Polyesters:					
Saturated	NA	4,000	10,000	16,000	25,000
Unsaturated	NA	4,000	8,000	10,000	15,000
UF/MF	5,000	8,000	12,000	15,000	18,000
PF-Moulding	5,000	7,500	10,000	12,500	15,000
Epoxy	NA	1,000	2,000	4,000	7,000
Miscellaneous:					
CA/CAB	NA	2,500	3,500	4,500	6,000
Polyacetal/PTEF/ PPO	NA	1,000	5,000	10,000	20,000
Total		34,600	78,000	118,350	173,500

Table - 6: Consumption of organotin compounds (Million Pounds).

Year	PVC Heat Stabilizers	Biocides	Polyure-thane Catalysts	Animal Products	Miscella-nious
1977	17.6	5.0	2.1	0.3	1.0
1978	19.8	5.5	2.4	0.3	1.0
1979	22.5	6.0	2.5	0.3	1.1
1980	20.2	5.8	2.1	0.3	1.0
1981	21.3	5.9	2.3	0.3	1.1
1982	19.2	5.5	2.1	0.3	1.0
1985	23.5	7.4	2.5	0.3	1.1
1990	33.0	12.0	3.5	0.4	1.3

Source : Morden Plastics, September issues & MRI estimates (12).

Table - 7: Organotin Toxicity

Toxicity	Alkyl tin form and Organic Constituent		
	Trialkyl	Dialkyl	Monoalkyl
Neurotoxicity (CNS)	Me Et	Bu	None
Hepatotoxicity (Biliary Tract)	Bu	Ethyl Propyl Butyl Pentyl	None
Immunotoxicity (T-cell)	None	Butyl Octyl	None
Mutagenesis/Carcinogenesis	Equivocal data	Insufficient data	Insufficient data
Skin/Eye Irritant	Me Et Bu	Me Propyl Bu Octyl	None

Table - 8: Acute Toxicity of Selected tin Compounds in Mammals*

Tin Compound		Animal	Route	LD_{50} (mg/kg)
Inorganic tin:	Stannous Chloride	Mice	Oral	250
R_4Sn:	Tetramethyltin	Rat	Oral	195-331
	Tetraethyltin	Rat	Oral	6-9
	Tetrabutyltin	Rat	Oral	4000
	Tetraoctyltin	Rat	Oral	50,000
R_3SnX:	Trimethyltin acetate	Rat	Oral	9.1
	Triethyltin acetate	Rat	Oral	4.0
	Triethyltin acetate	Rat	Oral	5.0
	Tributyltin chloride	Rat	Oral	129
	Trioctyltin chloride	Rat	Oral	4,000
R_2SnX_2	Dimethyltin chloride	Rat	Oral	73.9
	Di-n-butyltin Dichloride	Rat	Oral	100
	Dioctyltin dichloride	Rat	Oral	4,000
$RSnX_3$	Butyltin trichloride	Rat	Oral	2200-2395
	n-octyltin trichloride	Rat	Oral	4600
Stabilizers	Dibutyltin dilaurate	Rat	Oral	175
	Dibutyltin dimono-butyl maleate	Rat	Oral	120
	Di-n-octyltin thio-glycolate	Rat	Oral	945
	Di-n-octyltin maleate	Rat	Oral	1265
	Di-n-octyltin	Rat	I.P.	95
	dilaurate	Rat	Oral	6450

* Data of Piver (10), Smith (39), Stoner et. al. (40), Robinson (41), Klimmer (42), Pelikan, et. al., (43), and Aldridge, et. al. (44).

Table - 9: Signs and Symptoms of TMT and TET Poisoning in Humans:

TRIETHYLTIN (TET)	TRIMETHYLTIN (TMT)
Headache	Headache
Abdominal pain	Pain (generalized)
Visual disturbances	Visual Disturbances
Vertigo	Disorientation
Weight loss	Loss of Appetite
Hypothermia	Memory Deficits
Paralysis	Sleep Disturbances
Papilloedema	Loss of Libido
	- Bouts of Depression
	- Attacks of Rage

Source: Barnes and Stoner (32)

Sources: Fortemps et al., (46) and Ross et al., (47).

Table - 10: Behavioral Changes caused by triethyltin in mammals:

Behavioral indices/ species	Route of administration & duration	Observation	References
Motor Activity (Mouse)	2 mg/kg; ip (27 days)	Increased	(93)
(Rat)	1.5 or 3.0 mg/kg; sc (1 dose)	Increased	(89)
	5 or 10 ppm; water (3 weeks)	Increased	(89)
Startle Response	5 or 10 ppm; water (3 weeks)	Amplitude reduced	(89)
(Rat)	1 or 2 mg/kg; po	Amplitude reduced	(95)
Landing Foot-Spread (Rat)	5 or 10 ppm; water (3 weeks)	Reduced	(89)
Grip Strength (Rat)	1 or 2 mg/kg; po (3 doses)	Reduced	(96)
Response to pain (Rat)	0.25 or 0.5 mg/kg sc (14 doses)	Impaired	(99)
Schedule-controlled performance(Rat)	0.5, 1.0 or 1.5 mg/kg; ip (4 doses)	Rates reduced	(91)
Flavour Aversion (Rat)	1 or 3 mg/kg; po (4-5 doses)	Positive	(92)
	0.375, 0.75, 1.5 or 3.0 mg/kg; ip (2 doses)	Positive	(100)
Active Aboidance (Mouse)	2 mg/kg; ip	Not affected	(93)

Table - 11: Behavioral changes caused by trimethyltin in mammals:

Behavioral indices/ spices	Route of administration and duration	Observation	References
Motor Activity (Rat)	7 mg/kg; po (1 dose)	Hyperactivity	(95)
	5.6 or 7 mg/kg; po (1 dose)	Altered pattern of hyperactivity	(101)
Radial Arm Maze (Rat)	6 mg/kg; po (1 dose)	Accuracy decreased	(96)
Schedule-controlled performance (Rat)	7 mg/kg; po (1 dose)	Rates increased	(95)
Passive avoidance (Rat)	5, 6 or 7 mg/kg; po (1 dose)	Retention impaired	(102)
Hebb-Williams Maze (Rat)	7 mg/kg; po (1 dose)	Error increase	(97)
Alcohol Selection (Rat)	7 mg/kg; po (1 dose)	Preference reduced	(103)
Flavour Aversion (Rat)	0.625, 1.25, 2.5 or 5.0; ip (1 or 3 doses)	Positive	(100)
Mouse	3 mg/kg; ip (1 dose)	Altered hypoactivity	(98)
Mouse	3 mg/kg; ip (1 dose)	Rate reduced Altered Pattern	(98)

Table - 12: Rabbit Muscle Tissue Response to Implanted PVC Formulations Containing Organotin Compound.

Organotin in PVC +	Tissue Response	
	One Week	One month
DBT diisooctyl maleate	P	N
DBT dilauryl mercaptide	P	N
DBT diisooctyl thioglycolate	N	N
Dioctyltin beta mercaptic propionate	P	N
PVC + 40% Plasticizer + 1.5% organotin	P	P
Poly ethylene (Control)	N	N

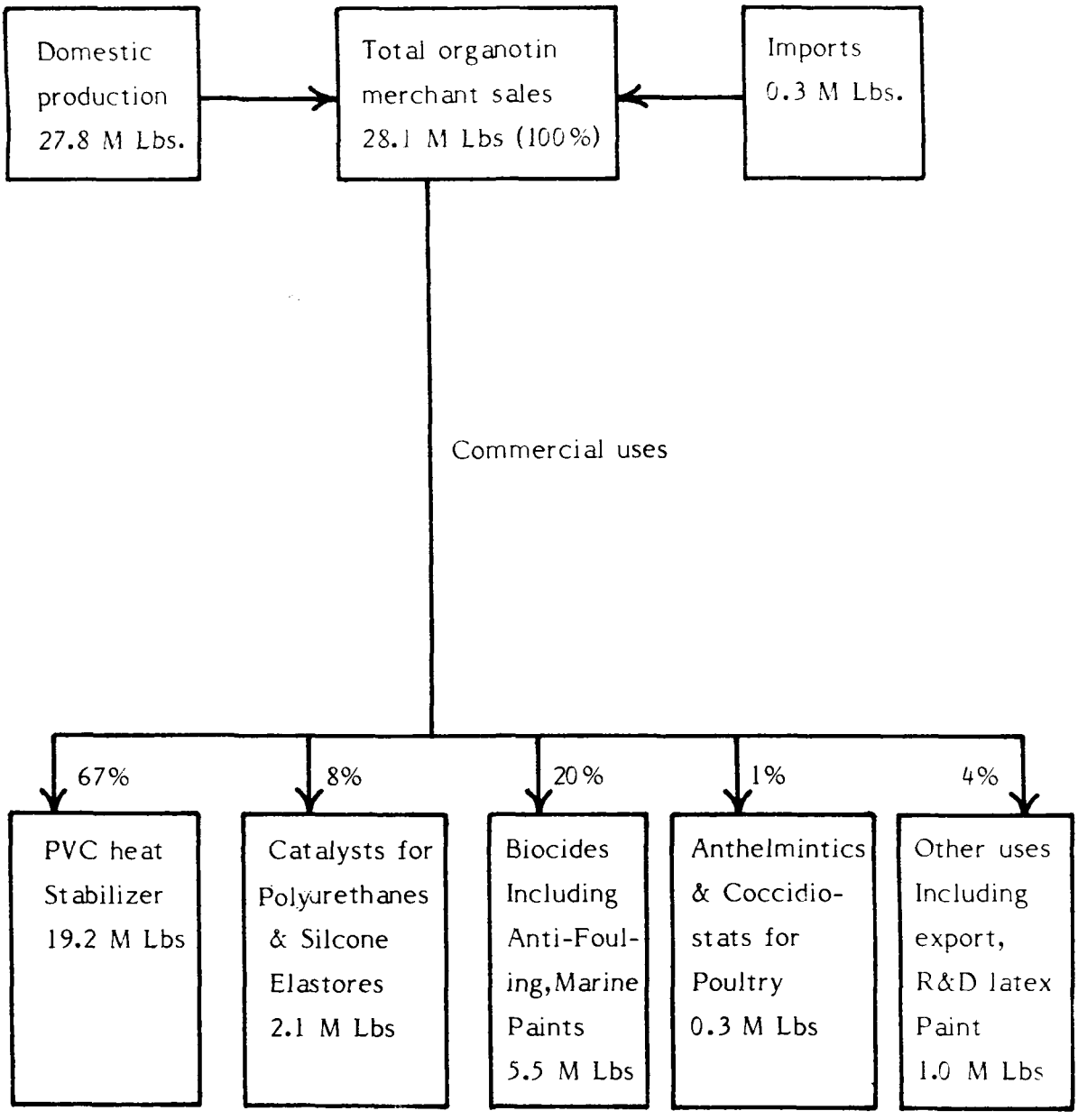


Fig. '1' : Illustration of the areas of applications of organotin compounds.

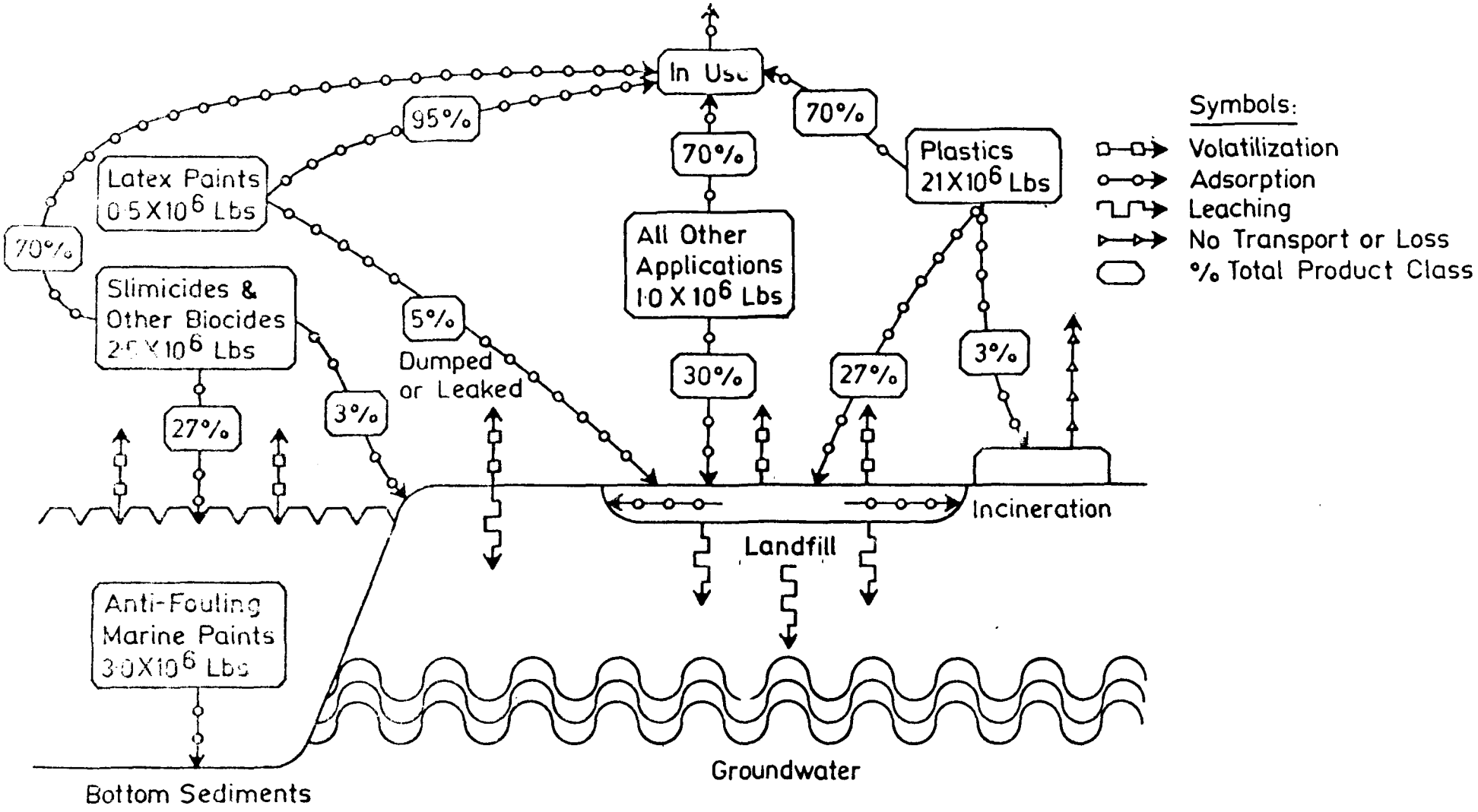


Fig:2 Environmental exposure of organotin

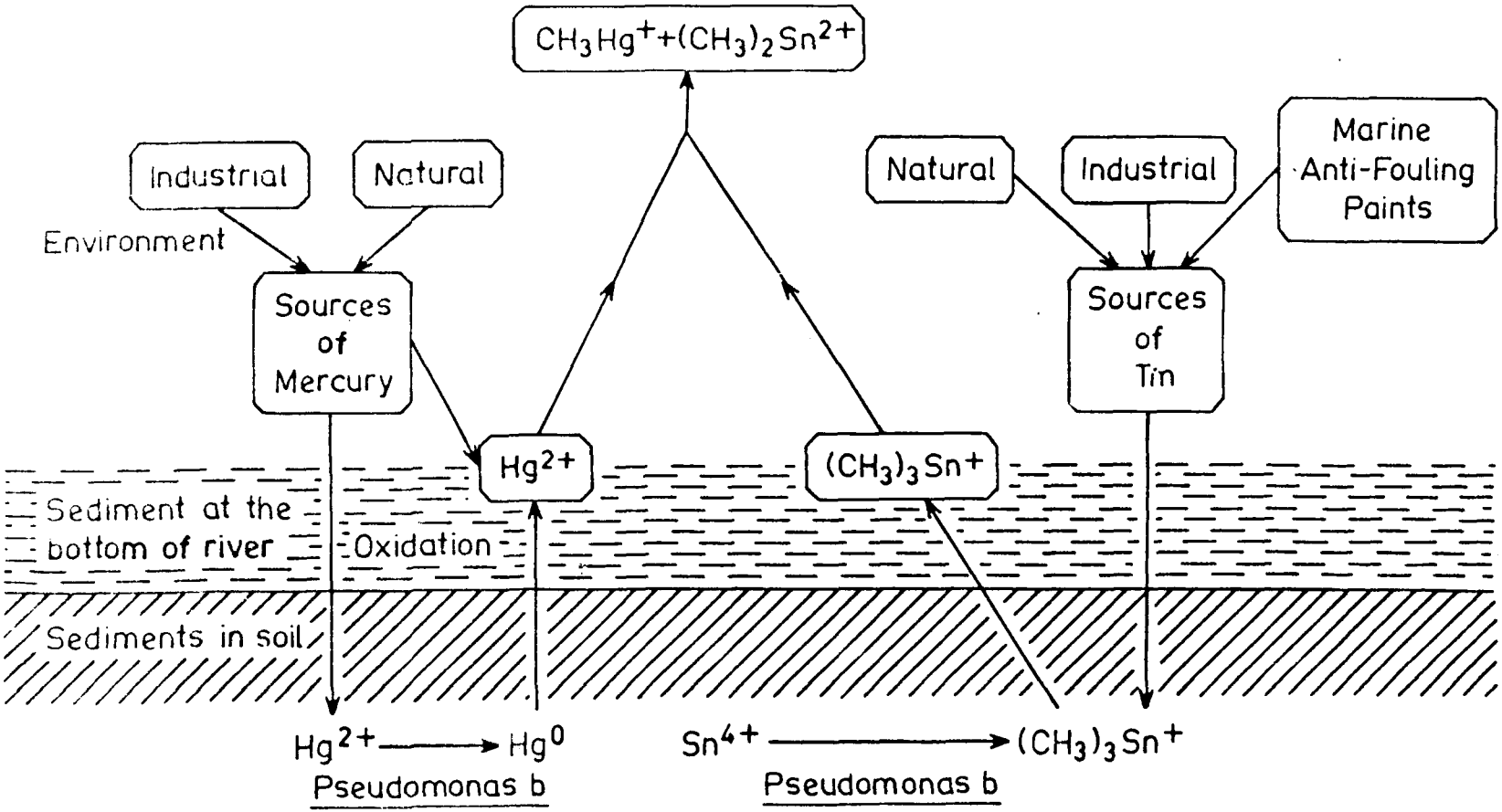
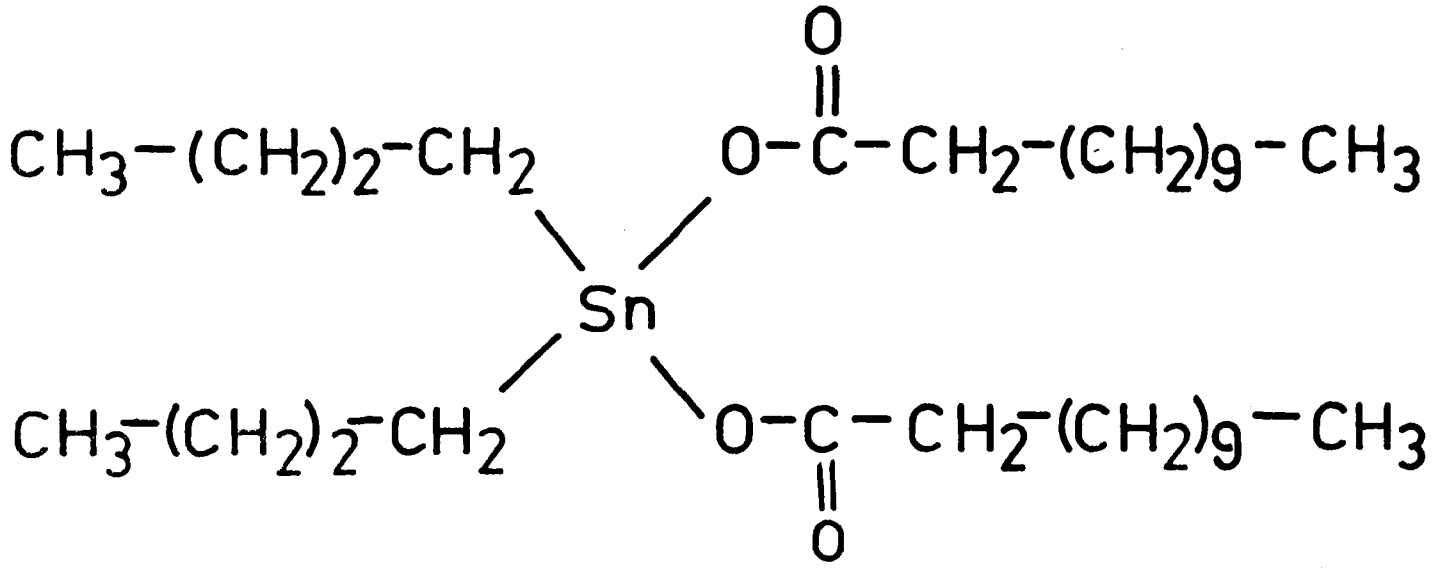


Fig:3 Bimetallic Mercury-tin "Crossover" Scheme.

Fig.4: STRUCTURE OF DIBUTYLTIN DILAURATE



CHAPTER - 1: SAFETY EVALUATION OF PLASTIC MATERIALS BEING INCREASINGLY USED IN THE STORAGE, PACKAGING AND DELIVERY OF FOOD, DRINKING WATER, COSMETICS AND LIFE SAVING FLUIDS.

INTRODUCTION:

Application and production of plastic materials are rapidly increasing in our country. Finished plastics are genrally considered to be safe, if they are used properly and manufactured using chemicals recommended by national and international regulatory agencies. However, it has been reported that some chemicals such as phthalate esters, organotins, metallic salts, and unreacted monomers such as acrylonitrile, styrene and vinyl chloride leach out during their use, which could be harmful for the consumers in the long term as some of these are toxic. Materials stored in plastics have been found to get coloured and impart odour due to migration of some chemicals. Ladies using plastic sticker (Bindi) have been found to develop skin irritation at the sites of the application. During recent years some small scale and to a certain extent few large scale industries are formulating plastic products by recycling the used plastics. These plastics are usually not tested by the regulatory agencies.

Different countries depending upon their requirements have laid down safety evaluation tests for the plastics to be used for the storage, packaging and delivery of food, drinking water, cosmetics and life saving fluids and drugs. British Plastic Federation (BPF), U.K., National Formulary (NF), U.S.A., Pharmacopoeia of Japan (Pharm. J.)., Food and Drug Administration (FDA), U.S.A., British Pharmacopoeia in other countries and Bureau of Indian Standards (BIS) New Delhi in our country have laid down certain standards/guidelines and provided protocols for the safety evaluation of plastics. BIS has recommended only the test of the global migration, in which certain compounds which are not volatile upto 95°C such organometallic compounds, phthalates, heavy metals etc., may be present, and the determination of the residual monomers such as vinylchloride and styrene in the plastic materials used in the biomedical devices, food packaging and drinking water (172-174). These monomers are not crosslinked with the

polymer and migrate out into the stored material.

To evaluate the safety of plastics available in the market, being used in large amounts, for the storage, packaging and delivery of food, drinking water, cosmetics and life saving fluids, the candidate has developed a test protol based on the above guidelines. The protocol used in this study for the safety evaluation of plastic materials includes test for change in pH of the extracts and presence of colour, odour, u.v. absorbing materials, oxidisable matters and heavy metals in the extract of plastics in addition to the test recommended by BIS. Presence of heavy metals in the plastics and residue on ignition of plastics were also performed. These additional parameters were included in view of the fact that the use of certain toxic metallic additives, antioxidants and UV absorbers have been permitted in the plastic formulations used in packaging of food, pharmaceuticals and drinking water (120 & 175-180). Previous studies conducted at ITRC has shown leaching of toxic metals, oxidisable matters and UV absorbing materials in amounts above than the permissible limit from commonly used plastics (111, 112, 119). Some of the leachable additives of plastics such as derivatives of benzophenone, benzotriazole and amines which give characteristic absorption in the UV region and used as UV absorbing materials are known to produce skin irritations and even carcinogenisity (182-183). In order to completely rule out the carcinogenic risk, the test for the UV absorbing materials have also been performed.

Equipments Used:

1. Hot air IEC Laboratory oven, capable of maintaining the desired temperature ±0.5°C, throughout the test period.
2. Elite heating mentle, equiped with temperature regulator.
3. Colourless laboratory glass wares, including Vetrosil crucibles.
4. Mettler H-35AR balance, with a sensitivity of 0.001 mg.

5. Heating Furnace, capable of maintaining temperature upto 600°C.
6. Perkin Elmer 5000 Atomic Absorption Spectrophotometer.
7. Pye Unicam SPS-200 spectrophotometer.

Plastic Samples:

Safety evaluation of about 600 samples (35 brands) of commonly used plastic materials viz. water tumblers, freeze bottles, water containers and lunch boxes each of four different makes referred as A,B,C and D brands., baby feeding bottles, tubes for the storage of cosmetics each of three different makes referred as A, B and C brands., Mugs, Jars, sheets, transfusion pouches and blood bags each of two different makes referred as A & B brands and dialysis set, powder containers and sticker bindi each of one brands were performed.

Procedure:

The samples were examined for any cracks or holes. The brands and names of the manufactures were also recorded. Out of 35 brands of the sample tested in this study, 14 brands were tested according to the recommendation given by BIS and the remaining 21 brands according to the test protocol developed at ITRC based on the recommendation of Bureau of Indian Standards, British Plastic Federation, British Pharmacopoeia, Pharmacopoeia of Japan, National Formulary, and Food and Drug Administration.

Plastic areas free from printing materials were used for the extraction studies. Plastic materials were cut into small pieces (5x0.5 cm) and contacted with the extractants in the ratio of 1 cm^2 plastic/2 ml extractants, according to BIS recommendations and 5 cm^2 plastic/ml extractants according to the recommendations of B.P.F., and kept in a hot air oven at a particular temperature for particular duration. About 600 cm^2 surface area were used of the plastic materials tested according to BIS recommendations and 3000 cm^2 surface area were used according to the recommendations of British Plastic Federations.

EXTRACTANTS

1.	Distilled water	-	Redistilled Water prepared by the ion exchange method.
2.	Acetic Acid	-	3% (m/v) prepared in distilled water.
3.	Acetic Acid	-	5% (m/v) prepared in distilled water.
4.	Ethyl Alcohol	-	5% (w/v) prepared in distilled water.
5.	Ethyl Alcohol	-	8% (v/v) prepared in distilled water.
6.	Sodium carbonate	-	5% (m/v) preapred in distilled water.

Test Conditions

1.	40°C for 24 hours	-	Plastics intended for the long term contact with food, pharmaceuticals, and drinking water at lower temperature (172).
2.	60°C for 10 days	-	Plastics intended for the long contact with food, pharmaceuticals and drinking water at higher temperature (136).
3.	60°C for 2 hours	-	Plastics intended for the brief contact with food and water at higher temperatures (172).
4.	70°C for 24 hours	-	Plastics intended for long and brief contact with pharmaceuticals (181 & 182).

Physico-chemical Tests:

The extracts obtained under the above conditions were examined for clarity of colour, odour and turbidity and subjected to tests for global migration, UV absorbing materials, heavy metals and oxidisable matters. The residue on ignition of plastics and heavy metals content in the plastics were also determined in the residue of 1 gm plastic.

Heavy Metals:

Plastic extracts (10 ml) were digested with 1.0 ml concentrated HNO_3 and metal contents were analysed using Perkin-Elmer 5000 - Atomic Absorption Spectrophotometer. Each extract was analysed in quadriplicate for the presence of metals. Results were expressed in ppm.

Global Migration:

Plastic extracts (100 ml) were evaporated in a constant weighed crucible in an oven maintained at a constant temperature (105°C or 95°C). In parallel corresponding extractants were also evaporated in identical manner. The difference in weight of the extract and the extractants was taken as the measure of the global migration. The test was performed in quadriplicate, and the results were expressed as global residue in mg/100 ml extract.

UV Absorbing Materials:

The UV absorbing materials were determined spectrophotometrically by scanning the extracts between 220 nm to 400 nm. The test was performed in quadriplicate and results were expressed in O.D.

Oxidisable Matters:

The oxidisable matters were determined by the titration of extract and corresponding blank with sodium thiosulphate. To 20.0 ml of plastic extracts, 20 ml of $KMnO_4$ (0.01 N) and 1 ml H_2SO_4 (2 N) were added. The contents were boiled. After cooling the contents, 1 ml of KI (0.1 gm/ml) and 5 drops of starch solution (saturated) were added. The solution was titrated against 0.01 N $Na_2S_2O_3$ till the pink colour disappeared. Similarly the blank solution was also titrated. The difference in the volume of $Na_2S_2O_3$ consumed in the titration of extract and blank was taken as the amount of oxidisable matters. The test was performed in quadriplicate. The results were expressed as ml of $Na_2S_2O_3$ utilized.

Residue on Ignition and Heavy Metals in the Residue:

The total residue content of the plastic was measured by burning one gram of plastic materials in a muffle furnace at 600°C to a constant weight. The results were expressed as mg of residue/gm plastics. The residue obtained was also used for the determination of metals. The residue were digested with 2N, HCl and made up to 10 ml with distilled water. The metal content in the residue was analysed as described above. The test was performed in quadriplicate.

Biological Tests:

Groundnut oil, distilled water and normal saline extracts were used for the biological tests. Healthy male mice weighing 20 ± 2 gm were used. Ten mice were given 0.5 ml normal saline extract intravenously (i.v.) and 10 mice were given 1 ml groundnut oil extract of the plastic intraperitoneally (i.p.) and 10 mice received the extracts of distilled water orally. Equal number of control mice were injected with normal saline, groundnut oil or distilled water in an identical manner to serve as corresponding controls. At 4, 24, 48 and 72 hours after the treatment, animals were examined for the mortality or any other toxicity symptoms. The test was repeated twice and was deemed to be satisfactory if all the animals survived for 90 hours and showed no signs of gross toxicity. The biological tests were conducted only on the plastics used in biomedical devices.

Estimation of unreacted monomer (styrene) in the plastics(173):

Apparatus used:

a. **Gas liquid chromatograph**

b. **Detector:** A flame ionization detector.

c. **Attenuator:** The instrument equipped with a multistep attenuator to ensure maximum peaks from the detector output signal and kept within the recorder chart range.

d. **Oven:** When operating isothermally, the oven was capable of maintaining test temperature to an accuracy of ± 0.3°C during the time for which the test sample and the corresponding reference standard were analysed.

e. **Gas flow regulator:** The metering mechanism was capable of maintaining flow rates constant upto ± 0.5 percent during the time in which the test sample and the corresponding reference standards were analysed.

f. **Recorder:** A one mv 250 mm strip chart recorder with a full scale response time of one second and a minimum chart speed of 12.5 mm/min.

g. **Clolumn:** A 6 feet length and 2 mm I.D. glass column filled with 5% OV-17 on W-HP 100/120 mesh.

h. **Sample injection:** Samples were injected with a 10 ul hamilton syringe which had sufficient precision on successive additions of the same test sample so as to produce recorder responses with variations not more than 1% on peak heights.

i. **Shaker:** Wrist action type.

j. **Reagents:** Methylene chloride, dimethyl formamide, and carbon bisulphide had been found to be satisfactory as they were capable of completely dissolving the polymer, allowing column separation and did not interfere in the analysis at the desired temperature.

k. **Carries gas:** Nitrogen gas of commercial grade were used.

Conditions of column temperature (100°C) and carrier gas flow (25 ml/min) were selected to produce adequate separation in the minimum amounts of time. Accurately four standards of styrene (1, 5, 10 and 100 ug/ml) diluted in methylene dichloride were preapred, introduced into the column and were allowed

for completely elution. From standard chromatograms, mass versus peak height curves were preapred. Standards were run each time if changes in the operating conditions were made to ensure no significant drift in the instrument.

Procedure:

Twenty brands of plastic samples used for the storage, packaging and delivery of food, drinking water and life saving fluids were purchased from local market. The surface area free from printing materials were used for the determination of unreacted styrene. The plastic samples were washed thoroughly with distilled water, cut into small pieces, weighed to about two grams and refluxed in dimethyl formamide till the polymer got completely dissolve, precipitated with methanol and finally filtered. The filtrate was evaporated to dryness and made up with dimethyl formamide to 10 ml. The samples thus prepared were injected at the GLC column, which was set at the same conditions at which the standard was analysed. After complete elution, the peak of the samples obtained were compared with the peak of the standard and the contents of styrene present as unreacted monomer in the plastics were determined.

Statistical Analysis:

For heavy metals, and global migration, statistical analysis were also made. Each samples was analysed in quadriplicate and data were evaluated by the student's 't' test. A value of $P<0.05$ was considered to be significant (183).

Results and Discussions:

The requirements/permissible limits and the summary of the results of physico-chemical tests performed on extracts of various plastic samples are shown in Table 13. As evident from the table, water tumblers of brands A and D, mugs of brand A, water containers of brand A, B and C, lunch boxes of brand D and sticker bindi showed leaching of colour in the extracts. In the extracts of water tumblers of brands A and C, freeze bottles and water containers of brands A and D, plastic type odour was present. Out of 35 brands of the samples tested, 21 brands showed leaching of UV absorbing material and global migration in concentrations above than the permissible limit. Out of the 21 brands of plastic samples subjected for the test of oxidisable matters, 12 brands showed leaching of oxidisable matters in concentrations above than the permissible limit.

Migration of heavy metals was studied in all brands of plastics. Leaching of heavy metals such as Pb, Cd, Mn, Cr, Cu and Zn in one or more extracting media was found to be in amounts above than the permissible limit, in 26 brands of plastics.

Twenty brands of samples were studied for the estimation of styrene present as unreacted monomer. Out of twenty brands of plastic, only in six brands styrene was detected and in two brands it was found to be above than the permissible limit (173).

It is interesting to note that all the samples tested in tihs study, showed residue on ignition above than the permissible limit except, baby feeding bottles of A and C brands, blood bags of B brand and tubes for the storage of cosmetics of A and B brands (Table 14).

About 57. % of the total samples tested as per the guide-line of BIS did not meet the prescribed limits, while about 97. % of these samples were not found to meet the requirements/permissible limits laid down by other international regulatory agencies.

The results of the biological tests were found to be satis-factory for all the brands of blood bags and transfusion pouches/dialysis set tested as no mortality or overt toxicity symptoms in the group of animals exposed to the plastic extracts/extractants were noticed.

Our results suggest that most of the plastic materials available in the market did not meet the safety evaluation tests for the plastic materials. Their tests should be performed regularly. The manufacturers of the plastic products should ensure before introducing them into the market that the materials manufactured meet the requirements as per their utility.

Proper precautions during their uses are essential and should be used only for those purposes for which they have been designed and tested. The possibility of the health hazards to the consumers of plastic exists, if non-food grade or untested plastics are used, since some of theleachable additives have toxicogenic potential.

The acute biological tests performed with the extracts of plastic materials suggest that these articles are not likely pose any health hazards. However, the consumers of the plastic may be at risk on the long term basis, due to the migration of various injurious chemical additives from plastic materials as some of these have also been detected in the environments, animal and human tissues.

Table - 13 Requirements/Permissible Limits for Plastic Materials Tested and Summary of the Results of the Tests Performed.

Tests performed		Requirements/Permissible Limits	Plastic Samples Unable to meet the requirements/permissible limits
Colour of Extracts	:	No. colour	Water tumblers (A & D), Mugs (A), Water Containers (A,B,C,), Bindi Sticker and Lunch boxes (C)
Odour of Extracts	:	No odour	Water tumblers (A,C), Freeze bottles (A,D), Water containers (A,D)
UV absorbing materials	:	0.3 O.D.	Water tumblers (A), Mugs (A), Freeze bottles (A,C,D), lunch boxes (A,B,C), water containers (A,B,D), Baby feeding bottles (B), Sheets (A), Jars (A,B,), Tubes (A,B,), Bindi, Blood transfusion pouch (A), Jugs (A,B).
Global migration	:	5 mg/100 ml extracts	Water tumblers (A,B,C,), Mugs (A,B,), Freeze bottles (A,B,D), Lunch boxes (A,B,C), Baby feeding bottles (A,B,C), Sheets (A), Jars (A,B,), Bindi sticker, water containers (A,B,D).
Oxidisable matters	:	Difference in volume of 0.01 N $Na_2S_2O_3$ should not be more than 2 ml during titration in comparison with blank.	Water tumblers, Mugs (A), Freeze bottles, Lunch boxes, Water containers (C), Baby feeding bottles (A), Sheets (A,B), Jars (A,B), Blood Bags (A), Transfusion pouches (B).

Contd.....Table 13

Heavy metals	:	Cu, Cr, Zn, Mn and Pb shold not exceed 1 ppm in the Extract and Cd should not exceed 0.1 ppm in the Extract	Water tumblers (A,B,C,), Mugs (B), Freeze bottles (A,B), Lunch boxes (A,D,), Water containers (A,B,C,D), Baby feeding bottles (B,C), Sheets (A), Jars (A,B), Cream tubes (A,B,C), Bindi Sticker, Transfusion pouches (A,B), Dialysis set, and Blood Bags (A&B)
Residue on Ignition	:	1 mg/gm plastic	Except Baby feeding bottles of A & C brands, blood bags of B brand, Tubes of A & B brands, all the samples tested.
Heavy metals in the residue	:	Metal content should not be more than 5 ppm in the ash of 1 gm plastic	Water tumblers (A,D), Water containers (A,B,D), Lunch boxes (D), Baby feeding bottles (B)
Unreacted styrene	:	The total residual styrene, when present, should not exceed 0.2 percent by mass of the polymer.	Blood bags (A) and Dialysis set.

Table 14: Articles Showing Residue on Ignition and Heavy Metals in the Residue of 1 gm Plastic.

Articles	Residue on Ignition in mg/gm plastic	Heavy Metals in the Residue in ppm
Water tumbler 'A'	6.9*	Cd (2.0), Mn (4.8), Cu (5.3)*, Cr (4.9)
Water tumbler 'B'	3.4*	Cd (0.7)
Water tumbler 'C'	5.8*	Cd (0.95), Cu (3.82), Zn (3.29)
Water tumbler 'D'	4.3*	Pb (9.41)*, Zn (4.39), Cd (3.04)
Plastic Mug 'A'	6.22*	Cd (0.3)
Plastic Mug 'B'	3.91*	Cd (0.4), Cr (2.3)
Water container 'A'	11.4*	Zn (22.24)*
Water container 'B'	3.4*	Pb (16.84)*, Cd (0.4)
Water container 'C'	1.9*	Zn (2.4), Pb (2.6), Cd (0.49)
Water container 'D'	10.2*	Zn (9.88)*, Cd (0.69), Cu (2.70)

Permissible limit (PL): Residue on Ignition. The weight of ash of 1 gm plastic should not be more than 1 mg; and the concentration of metals in the ash should not be more than 5 ppm.

: Values are mean of four samples.

* : Above the permissible limits.

Contd.....Table 14:

Freeze bottle 'A'	2.5*	Cd (1.2), Cr (2.8), Mn (2.8)
Freeze bottle 'B'	2.8*	Cd (2.8), Pb (3.5)
Freeze Bottle 'C'	3.2*	Cd (0.4)
Freeze bottle 'D'	1.9*	Zn (3.2), Pb (2.2), Cd (0.8), Mn (2.5)
Lunch box 'A'	3.5*	Cd (2.1), Pb (3.8), Cu (3.1), Mn (1.9)
Lunch box 'B'	3.2*	Cd (1.2)
Lunch box 'C'	1.9*	Zn (1.36)
Lunch box 'D'	6.0*	Zn (5.2)*
Baby feeding bottle 'A'	0.63	Cd (0.2)
Baby feeding bottle 'B'	1.98*	Pb (4.1), Zn (5.2)*
Baby feeding bottle 'C'	0.98	Cd (0.2), Zn (2.4)
Plastic sheet 'A'	2.8*	Cd (3.0), Zn (4.8)
Plastic sheet 'B'	1.2*	Cd (0.30)
Plastic Jar 'A'	1.9*	Cd (0.8), Pb (2.79)
Plastic Jar 'B'	3.2*	Cd (0.85), Pb (2.92)

Contd.....Table 14

Plastic tube for shampoo (A)	0.80	Cd (0.8), Zn (2.0)
Plastic tube for face cream (B)	0.90	Cd (0.73)
Plastic tube for make up lotion (C)	1.8*	Cd (1.0)
Plstic container for face powder	1.9*	Cd (0.85)
Plastic bindi	12.20*	Pb (3.0), Zn (2.8)
Transfusion pouch 'A'	3.8*	Cu (3.8), Zn (2.59)
Transfusion pouch 'B'	0.95	Cr (4.0), Cd (0.28)
Dialysis set	0.90	Cd (0.84)
Blood bag 'A'	2.5*	Pb (2.8), Zn (4.2)
Blood bag 'B'	0.85	Cd (0.34)

* Above the permissible limits. Permissible limit (PL) - Residue on Ignition. The weight of ash of 1 gm plastic should not be more than 1 mg. Heavy metals in the residue. The concentration of metals should not be more than 5 ppm.

: Values are mean of four samples.

CHAPTER - 2: EFFECT OF SOME PHYSICO-CHEMICAL FACTORS SUCH AS pH, TEMPERATURE, SUNLIGHT, STORAGE TIME AND THE CHEMICAL NATURE OF THE EXTRACTANTS ON THE MIGRATION OF CHEMICAL ADDITIVES FROM FINISHED PLASTICS.

INTRODUCTION:

In our country the material stored in plastic pouches/containers in most cases have often to stand high temperature and sunlight. In rural area and also in some urban area plastic containers are used for the storage of pickles etc., which have an acidic pH and are exposed to sunlight for curing. Usually fruit juices, transfusion fluids, medicines, vegetable oils and some food preparations are stored in plastic pouches/containers for a longer period and in summer season exposed to high temperature during delivery. So far no specific guidelines for the safety evaluation of plastics under such conditions have been provided in our country. The leachability of the chemical additives could be modified by the physico-chemical factors and may render the materials harmful stored in plastic products. Therefore, in the present investigations, effect of sunlight, temperature, storage time, pH and aquous and alcoholic extracting media on the migration of chemical additives from finished plastics materials used for the storage, packaging and delivery of food, drinking water and life saving fluids have been studied.

MATERIALS AND METHODS:

The plastic lunch boxes, freeze bottles, water tumblers and blood bags each of two makes referred as brands A and B were purchased from local market. The plastic areas free from printing materials were used for the extraction studies. The plastic materials were cut into small pieces (5 x 0.5 cm) and washed thoroughly with distilled water and immersed into different extractants in the ratio of 1 cm^2 plastic/2 ml extractants (172).

Extracting Conditions:

a. 8 hours at 40-45°C.

b. 8 hours at room temperature (25°C).

c. 8 hours in the sunlight (40-45°C)

d. 24 hours at 40°C

e. 24 hours at room temperature (25°C)

f. 2 hours at 60°C

g. 2 hours at room temperature (25°C)

h. 240 hours at 60°C

i. 240 hours at room temperature (25°C).

The extraction duration for 8 hours at / room temperature was taken as the control for 8 hours at 40-45°C, 8 hours at 40-45°C as the control for 8 hours in sunlight, 24 hours at room temperature as the control for 24 hours at 40°C, 2 hours at room temperature as the control for 2 hours at 60°C and 240 hours at room temperature as the control for 240 hours at 60°C. The extracting conditions for 2 hours at 60°C and 24 hours at 40°C have been recommended by BIS (172). Whereas 240 hours at 60°C has been mentioned by British Plastic Federation (BPF) (135 & 182).

Extractants and Recommending Agencies:

a.	Double distilled water	BIS and BPF
b.	3% Acetic Acid	BIS
c.	5% Acetic Acid	B. Pha.
d.	5% Ethanol	BPF
e.	8% Ethanbol	BIS
f.	5% Sodium Carbonate	BPF
g.	0.9% Sodium Chloride	BIS, BPF and B. Pha.

Statistical Analysis:

Each sample was analysed in quadriplicate and data were evaluated by the student's 't' test. A value of $p < 0.05$ was considered to be significant (183).

The details of the test procedure and methodology have been mentioned in Chapter - 1.

RESULTS:

Influence of storage time on the migration of heavy metals such as Pb, Cd, Cr, Cu, Mn and Zn from plastic freeze bottles, water tumblers, lunch boxes and blood bags is shown in figures 5 and 6.

It is shown in Figure 5 that freeze bottles of brand 'A' showed migration of Cd in amounts above than the permissible limit in 5% sodium carbonate and that of Cd and Pb in 5% acetic acid when extracted for 240 hours, and none of the metals migrated above than the permissible limit when extracted for 2 hours, 8 hours and 24 hours. It is interesting in note that freeze bottles of brand B did not contribute to the leaching of any metals in concentration above than the permissible limit under the conditions of extractions and extractants used (Fig. 5). Water tumblers of brand A also did not contribute to the migration of any metals in amounts above than the permissible limit in any extracting media when extracted for 2 hours and 8 hours. However, when extracted for 24 hours only 5% acetic acid showed migration of Cd and Cu in amounts above than the permissible limit. When the extraction duration was increased from 24 hours to 240 hours, leaching of Cd occurred in concentrations above than the permissible limit in addition to 5% acetic acid, in distilled water, 3% acetic acid and 5% sodium carbonate also. It is interesting to note that migration of Cu was noticed in amounts above than the permissible limit in 8% ethanol. However, concentrations of Cu and Cd migrated in 5% acetic acid under the extraction duration for 240 hours, were higher than that obtained during 24 hours. Water tumblers of brand B also showed migration of only Cd in amounts above than the permissible limit in 5% acetic acid and 5% sodium carbonate when extracted for 240 hours.

Influence of storage time on the migration of heavy metals from plastic lunch boxes and blood bags of brands A and B is shown in Figure-6. As evident from the figure, lunch boxes of brand A did not contribute to the migration of any metals when extracted for 2 hours and 8 hours in any extracting media. However, migration of Cd occurred above than the permissible limit only in acidic medium when the extraction duration was increased to 240 hours. It is interesting to note that a further increase of extraction period from 24 hours to 240 hours contributed to the migration of Cd in concentration above than the permissible limit in distilled water and 5% sodium carbonate which was not seen during the extraction periods for 24 hours. Lunch boxes of brand B did not contribute to the migration of any metals in amounts above than the permissible limit during the extraction durations for 2 hours, 8 hours and 24 hours (Fig. 6). However, when the extraction period was increased to 240 hours migration of Cd was found in concentration above than the permissible limit in 5% sodium carbonate, Cu in 8% ethanol and of Cu and Cd in 5% acetic acid. Blood bags of brand A also did not contribute to the migration of any metals in concentrations above than the permissible limit during the extraction durations for 2 hours, 8 hours and 24 hours. However, when extracted for 240 hours migration of Cd occurred in amounts above than the permissible limit in 5% acetic acid and 5% sodium carbonate and of Cu in 8% ethanol. None of the metals migrated in amounts above than the permissible limit from blood bags of brand B under any extracting media and extracting conditions.

Effect of storage time on the migration of UV absorbing materials from plastic freeze bottles; water tumblers, lunch boxes and blood bags is shown in Figures 7-13.

As apparent from figure 7, freeze bottles of brand A showed migration of UV absorbing materials in detectable amounts but within the permissible limit only in 5% acetic acid and 5% sodium carbonate, when extracted for 2 hours and 8 hours. However, when the extraction period was increased to 24 hours, in addition to the above two extractants, 8% ethanol, 0.9% sodium chloride and 3% acetic acid also contributed to the migration of UV absorbing materials in detectable amounts. It is interesting to note that when the extraction period was further increased from 24 hours to 240 hours, UV absorbing materials leached out in detectable amounts in all the extracting media. The order of migration of U.V. absorbing materials in different extractants was : 5% acetic acid $>$ 5% sodium carbonate $>$ 3% acetic acid = 0.9% sodium chloride $>$ 8% ethanol $>$ 5% ethanol $>$ distilled water.

It is evident from Figure 8, freeze bottles of brand B did not contribute to the migration of UV absorbing materials when extracted for 2 hours in all the extracts. However, when the extraction duration was increased to 8 hours and 24 hours, 0.9% sodium chloride, 5% sodium carbonate and 3% and 5% acetic acid showed migration of UV absorbing materials in detectable amounts. When the extraction period was further increased to 240 hours, leaching of UV absorbing materials occurred in detectable amounts in addition to the above extracts in distilled water and 5% and 8% ethanol also. The migration of UV absorbing material in various extracts was found to be in the following order: 5% sodium carbonate $>$ 0.9% sodium chloride $>$ 5% acetic acid $>$ 3% acetic acid $>$ 8% ethanol $>$ 5% ethanol = distilled water.

Migration of UV absorbing materials from water tumblers of brand A is shown in Fig. 9. As evident from the figure, migration of UV absorbing materials, when extracted for 2 hours was found in detect-

able amounts only in 5% sodium carbonate. When the extraction period was increased to 8 hours and 24 hours in addition to 5% sodium carbonate, leaching of UV absorbing material was noticed in detectable amounts in 3% and 5% acetic acid and in 0.9% sodium chloride also. It is interesting to note that in sodium carbonate migration of UV absorbing material was above than the permissible limit when extracted for 24 hours. It is worthwhile to note that when the extraction period was further increased to 240 hours migration of UV absorbing material occurred in detectable amounts in all the extracts and 5% acetic acid and 5% sodium carbonate showed migration of UV absorbing material in concentrations above than the permissible limit. The order of migration of UV absorbing material in various extracts was: 5% sodium carbonate > 5% acetic acid > 0.9% sodium chloride > 3% acetic acid > 8% ethanol > 5% ethanol = distilled water.

It is shown in Figure 10, water tumblers of brand B showed migration of UV absorbing material in detectable amounts during the extraction for 2 hours in 5% sodium carbonate and 5% acetic acid. However, when the extraction period was increased to 8 hours and 24 hours in addition to the above extracts, 0.9% sodium chloride also showed migration of UV absorbing materials in detectable amounts. It is interesting to note that when the extraction period was further increased to 240 hours migration of UV absorbing materials occurred in detectable amounts in all the extracts except in distilled water, where the migration was nil, and in 5% sodium carbonate the migration of UV absorbing materials was found to be above than the permissible limit. Migration of UV absorbing materials in various extracts was in the following order: 5% acetic acid > 3% acetic acid > 5% sodium carbonate > 0.9% sodium chloride > 8% ethanol > 5% ethanol > distilled water.

As evident from Figure 11, lunch boxes of brand A showed migration of UV absorbing materials in detectable amounts only in 3% and 5% acetic acid during the extraction studies for 2 hours. When the extraction period was increased to 8 hours and 24 hours in addition to the above extracts, 0.9% sodium chloride and 5% sodium carbonate also contributed to the migration of UV absorbing materials in detectable amounts. It is interesting to note that when the extraction period was further increased to 240 hours migration of UV absorbing materials in detectable amounts were noticed in 8% ethanol and 3% and 5% acetic acid. In 5% sodium carbonate, migration of UV absorbing materials was found to be in concentration above than the permissible limit. Maximum migration of UV absorbing material was found in 5% acetic acid and order of migration in various extracts was: 5% acetic acid > 3% acetic acid > 5% sodium carbonate > 0.9% sodium chloride > 8% ethanol > 5% ethanol = distilled water.

It is shown in Figure 12, lunch boxes of brand B contributed to the migration of u.v. absorbing materials in detectable amounts only in 3% & 5% acetic acid and 5% sodium carbonate during the extraction for two hours, 8 hours and 24 hours. When the extraction durations was increased from 24 hours to 240 hours, migration of UV absorbing materials in detectable amounts occurred in all the extracts and in 5% acetic acid and 5% sodium carbonate it was above than the permissible limit. Migration of UV absorbing materials in the various extracts was in the following order: 5% sodium carbonate > 5% acetic acid > 3% acetic acid > 0.9% sodium chloride > 8% ethanol > 5% ethanol > distilled water.

Migration of UV absorbing materials from blood bags of brands A and B is shown in Fig. 13. As apparent from the figure, migration of UV absorbing materials were found to be nil in all the extracts during

the extraction periods for 2 hours and 8 hours. When the extraction period was increased to 24 hours and 240 hours UV absorbing materials leached out in detectable amounts in all the extracts except in distilled water, where the migration was not in detectable amounts. It is of great significance to note that the UV absorbing materials leached out was found to be within the permissible limit in all the extracts and the extracting conditions. Maximum migration of UV absorbing materials were noticed in 5% sodium carbonate and the order of migration in various extracts was: 5% sodium carbonate > 5% acetic acid > 3% acetic acid > 0.9% sodium chloride > 8% ethanol > 5% ethanol.

Influence of storage time on the global migration from plastic freeze bottles, water tumblers, lunch boxes and blood bags is shown in Figures 14-20.

As apparent from Figure 14, global migration from freeze bottles of brand A in detectable amounts was noticed only in 5% acetic acid and 5% sodium carbonate when extracted for 2 hours and 8 hours. When the extraction period was increased to 24 hours in addition to the above two extracts, 3% acetic acid and 0.9% sodium chloride also contributed to the global migration in detectable amounts and the increased rate of global migration was found to be significant in 5% acetic acid and 5% sodium carbonate in comparison with 3% acetic acid. When the extraction period was further increased to 240 hours, global migration in detectable amounts was noticed in all the extracts with an increased rate in 0.9% sodium chloride, 8% ethanol and 3% acetic acid which was significant with respect to distilled water. It is interesting to note that in 5% acetic acid and 5% sodium carbonate the increased rate of global migration was also found to be significant in comparison with 3% acetic acid. The order of global migration in various extracts was: 5% sodium

carbonate > 5% acetic acid > 0.9% sodium chloride > 3% acetic acid > 8% ethanol > 5% ethanol = distilled water.

As evident from Figure 15, the pattern of global migration obtained from freeze bottles of brand B, was found to be similar as in the case of freeze bottles of brand A (cf Figure 14), with the exceptions that when extracted for 240 hours, the global migration obtained was found to be in amounts above than the permissible limit in 5% sodium carbonate. The order of migration in different extracting media was: 5% sodium carbonate > 5% acetic acid > 0.9% sodium chloride > 3% acetic acid > 8% ethanol > 5% ethanol > distilled water.

As apparent from Figure 16, water tumblers of brand A did not contribute to the global migration in detectable amounts when extracted for 2 hours. However, when the extraction period was increased to 8 hours, 5% acetic acid and 5% sodium carbonate contributed to the global migration in detectable amounts. When extracted upto 24 hours in addition to the above extracts, 3% acetic acid and 0.9% sodium chloride also contributed to the global migration in detectable amounts. The increased rate of global migration in 5% acetic acid and 5% sodium carbonate was found to be significant in comparison with the migration obtained in 3% acetic acid. It is interesting to note that, when the extraction period was increased to 240 hours, global migration in detectable amounts was noticed in all the extracting media and in 5% acetic acid, 5% sodium carbonate and 0.9% sodium chloride it was above than the permissible limit. The increased rate of global migration in all the extracts was found to be significant in comparison with distilled water.

The order of global migration in various extracts was: 5% sodium carbonate > 5% acetic acid > 0.9% sodium chloride > 3% acetic acid > 8% ethanol > 5% ethanol > distilled water.

The gobal migration from water tumblers of brand B is shown in Fig. 17. As evident from the figure, the global migration was not found in detectable amount in any extracting media when extracted for 2 hours. However, after increasing the extraction durations for 8 hours and 24 hours, global migration in detectable amounts was found in 3% and 5% acetic acid, 0.9% sodium chloride and 5% sodium carbonate. it is worthwhile to note that when the extraction period was further increased to 240 hours global migration in detectable amounts was noticed in all the extracting media and was above than the permissible limit in 3% and 5% acetic acid and 5% sodium carbonate. The increased rate of global migration in all the extracts was found to be significant in comparison with the global migration obtained in distilled water. It is interesting to note that in 5% acetic acid the increased rate was also significant in comparison with the global migration obtained in 3% acetic acid. The order of migration in various extracts was: 5% acetic acid > 3% acetic acid > 5% sodium carbonate > 0.9% sodium chloride > 8% ethanol > 5% ethanol > distilled water.

It is shown in the Figure 18, the global migration from lunch boxes of brand A was not found in detectable amounts under any extracting media when extracted for 2 hours. When the extraction period was increased to 8 hours, 5% acetic acid and 5% sodium carbonate contributed to the global migration in detectable amounts. When extracted upto 24 hours in addition to the above two extracts, 3% acetic acid and 0.9% sodium chloride also contributed to the global migration in detectable amounts. The increased rate of global migration obtained in 5% sodium carbonate was found to be significant with respect to the migrations obtained in 3% acetic acid. It is interesting to note that when the extraction period was increased to 240 hours all the extracts showed global migration in detectable amounts and in 5% sodium carbonate it was

above than the permissible limit. It is worthwhile to note that the increased rate of global migration in all the extracts was found to be significant in comparison with the migrations obtained in distilled water. In 5% sodium carbonate the increased rate was also significant in comparison with 3% acetic acid. The global migration obtained in various extracts was found to be in the following order: 5% sodium carbonate > 5% acetic acid > 0.9% sodium chloride > 3% acetic acid > 8% ethanol > 5% ethanol > distilled water.

Pattern of global migration from lunch boxes of brand B is shown in Fig. 19. As apparent from the figure, the global migration was not found in detectable amounts when extracted for 2 hours. However, when the extraction period was increased to 8 hours and 24 hours, global migration in detectable amounts was noticed in all the extracts, except in the distilled water and 8% ethanol. It is interesting to note that when the extraction period was further increased to 240 hours all the extracts showed global migration in detectable amounts and in 5% acetic acid and 5% sodium carbonate the migration obtained was above than the permissible limit. The order of global migration in the various extracts was: 5% sodium carbonate > 5% acetic acid > 3% acetic acid > 0.9% sodium chloride > 8% ethanol > 5% ethanol > distilled water.

Global migration from blood bags of brand A is shown in Fig. 20. It is apparent from the figure, the global migration was nil in all the extractants when extracted for 2 hours and 8 hours. However, when extracted for 24 hours, 3% and 5% acetic acid, 5% sodium carbonate and 0.9% sodium chloride showed global migration in detectable amounts with an increased rate in 5% acetic acid which was significant in comparison with 3% acetic acid. It is interesting to note that when the extraction period was further increased to 240 hours all the extract showed global

migration in detectable amounts with an increased rate which was significant in comparison with distilled water, except 5% ethanol where significant increased rate of migration was not observed. It is worthwhile to note that the increased rate of global migration in 5% acetic acid and 5% sodium carbonate was also found to be significant with respect to 3% acetic acid. The order of global migration in different extgracts was: 5% sodium carbonate > 5% acetic acid > 0.9% sodium chloride > 3% acetic acid > 8% ethanol > 5% ethanol > distilled water.

Blood bags of brand B also did not contribute to the global migration in detectable amounts when extracted for 2 hours and 8 hours (cf Figure 20). However, when the extraction period was increased to 24 hours, 5% acetic acid and 5% sodium carbonate showed global migration in detectable amounts. When the extraction period was further increased to 240 hours, 3% and 5% acetic acid and 5% sodium carbonate showed global migration in detectable amounts with an increased rate of migration in 5% acetic acid in comparison with the migration obtained in 3% acetic acid. Order of global migration in different extracts was: 5% sodium carbonate > 5% acetic acid > 3% acetic acid.

Influence of sunlight on the migration of heavy metals from plastic freeze bottles, water tumblers, lunch boxes and blood bags is shown in Tables 15-21.

As evident from Table 15, freeze bottles of brand A showed migration of Cd above than the permissible limit under sunlight while at the same temperature in a hot air oven none of the metals migrated above the permissible limit when extracted with distilled water. When 5% and 8% ethanol and 0.9% sodium chloride were used as the extractants none of the metals leached out in amounts above than the permissible limit under any test conditions. It is interesting to note that when extracted

with 5% acetic acid and 5% sodium carbonate leaching of Cd occurred above than the permissible limit at both the test conditions, with a significant increased rate of migration of Cd under sunlight in comparison with the migration obtained in a hot air oven maintained at the identical temperature and duration.

It is shown in Table 16, freeze bottles of brand B showed leaching of Pb and Mn in amounts above than the permissible limit under sunlight, while none of the metals leached out above the permissible limit when extracted in a hot air oven at the same temperature in distilled water. When extracted with 8% ethanol none of the metals leached out above than the permissible limit in any test conditions and extracting media used. When 3% acetic acid was used as the extractant, leaching of Pb occurred above than the permissible limit in the hot air oven, while under sunlight including Pb, leaching of Mn and Zn was also noticed in concentration above than the permissible limit with a significant increased rate of Pb in comparison with the hot air oven maintained at the same temperature. When extracted with 5% acetic acid, leaching of Pb and Zn was noticed in the hot air oven and under sunlight including these two metals leaching of Mn was also found above than the permissible limit. The increased rate of Pb and Zn under sunlight was found to be significant in comparison with the migration obtained in the hot air oven. When extracted with 0.9% sodium chloride none of the metals leached out above than the permissible limit in the hot air oven, while leaching of Pb and Zn were noticed above than the permissible limit in sunlight. When the extraction studies were carried out with 5% acetic acid and 5% sodium carbonate migration of Pb and Cd was found to be above than the permissible limit under sunlight, while only Cd migrated when extracted in the hot air oven maintained at the same temperature and

duration. It is interesting to note that the increased rate of Cd was found to be significant under sunlight with respect to the migration obtained in a hot air oven maintained at identical temperature and duration.

As apparent from Table 17, water tumblers of brand A showed leaching of Cd above than the permissible limit in the hot air oven while under sunlight that of Cd and Mn was found in amounts above than the permissible limit. When extracted with 8% ethanol leaching of Cd and Cu was found above than the permissible limit under sunlight, while only Cd in the hot air oven. When extracted with 3% acetic acid leaching of again Cd was noticed in the hot air oven, while under sunlight including Cd, leaching of Mn and Cr was found in concentrations above than the permissible limit. The same metals leached out in 5% acetic acid under both the extracting conditions with a slight higher degree of migration than that obtained in 3% acetic acid. When 5% sodium carbonate and 0.9% sodium chloride were used as the extractants, migration of Cd was noticed inthe hot air oven, while under sunlight including Cd, leaching of Cr was also found above than the permissible limit. The increased rate of migration of Cd under sunlight was found to be significant in comparison with the migration of Cd in the hot air oven maintained at similar temperature and duration.

As evident from Table 18, water tumblers of brand B did not contribute to the leaching of any metals in amounts above than the permissible limit, either in the hot air oven or under sunlight when distilled water and 8% ethanol were used as the extractants. However, when extracted with 3% and 5% acetic acid and 5% sodium carbonate, leaching of only Cd occurred above than the permissible limit under both the conditions of extraction. While in 0.9% sodium chloride leaching of Cd above than the permissible limit was found only under sunlight. It is

interesting to note that the increased rate of migration of Cd under sunlight was found to be significant in comparison with the migration obtained in the hot air oven when 5% acetic acid and 5% sodium carbonate when used as the extractants.

As apparent from Table 19, lunch boxes of brand A showed migration of Cd and Pb above than the permissible limit in the hot air oven, while under sunlight including these two metals, leaching of Mn also occurred in amounts above than the permissible limit when distilled water was used as the extractant. In 8% ethanol none of the metals leached out in the hot air oven, while under sunlight leaching of Cu was found in amounts above than the permissible limit. When extracted with 3% and 5% acetic acid leaching of same metals occurred as were found in distilled water under both the extracting conditions. However, concentrations of all the metals leached out in acetic acid were found to be about 2 fold higher than that obtained in distilled water. When extracted with 5% sodium carbonate and 0.9% sodium chloride, migration of Cd was noticed in the hot air oven, while under sunlight migration of Pb also occurred in amounts above than the permissible limit. It is interesting to note that the increased rate of migration of Pb and Cd under sunlight were found to be significant in comparison with the migration obtained in a hot air oven under all the extracting media except ethanol, where the increased rate of metals leached out were not significant.

Migration of heavy metals from lunch boxes of brand B is shown in Table 20. As evident from the table migration of Cd and Zn were found to be above than the permissible limit under both the test conditions when distilled water was used as the extractant. Same metals leached out in 5% sodium carbonate also, however, an increased rate of migration with respect to distilled water were noticed in 5% sodium carbonate.

When extracted with 8% ethanol migration of Zn was found to be above than the permissible limit, while in 0.9% sodium chloride that of Cd was found. When extracted with 3% acetic acid, migration of Cd and Zn was found to be above than the permissible limit in the hot air oven, while under sunlight, including these two metals migration of Cr was also noticed in amounts above than the permissible limit. In 5% acetic acid same metals leached out under both the extracting conditions as was found in 3% acetic acid with the exceptions that Cr also leached out in the hot air oven. It is interesting to note that the increased rate of Cd under sunlight was found to be significant with respect to the migration obtained in the hot air oven when 0.9% sodium chloride and distilled water were used as the extractants and that of Cd and Zn when 3% and 5% acetic acid and 5% sodium carbonate were used as the extractants.

As apparent from Table 21, from blood bags of brand A none of the metals leached out in the hot air oven while under sunlight at same temperature leaching of Pb occurred in amounts above than the permissible limit in distilled water and 0.9% sodium chloride. When extracted with 8% ethanol none of the metals migrated in concentrations above than the permissible limit, under both test conditions. When 3% acetic acid and 5% sodium carbonate were used as the extractants, leaching of Zn was found in the hot air oven, while under sunlight migration of Zn and Pb both occurred in amounts above than the permissible limit. It is interesting to note that during the extraction studies with 5% acetic acid, migration of Pb & Zn both occurred above than the permissible limit under both the extracting conditions.

Effect of sunlight on the migration of heavy metals in one more brand of blood bags were studied. It was observed that none of

the metals leached out in amounts above than the permissible limit under the various extracting media and extracting conditions (data not shown).

Influence of sunlight on the migration of U.V. absorbing materials from plastic freeze bottles, water tumblers, lunch boxes and blood bags is shown in Figures 21-28.

As evident from the Figure 21, freeze bottles of brand A showed migration of U.V. absorbing materials within the permissible limit in distilled water and 8% ethanol under sunlight or in a hot air oven maintained at the same temperature. However, when 3% acetic acid and 0.9% sodium chloride was used as the extractants, the migration of U.V. absorbing materials were above than the permissible limit under sunlight and within the permissible limit when kept at the same temperature in a hot air oven. It is interesting to note that when 5% acetic acid and 5% sodium carbonate were used as the extractants, leaching of U.V. absorbing materials were above than the permissible limit in both the extracting conditions. The migration of U.V. absorbing materials in the various extracts were in the following order:

5% sodium carbonate > 5% acetic acid > 0.9% sodium chloride > 3% acetic acid > 8% ethanol distilled water.

As evident from Fig. 22, the migration of U.V. absorbing materials from freeze bottles of brand B were within the permissible limit in all the extracting media when kept under sunlight or at the same temperature in a hot air oven. However, an increased rate of migration of U.V. absorbing materials were noticed under sunlight, in comparison with the migration obtaioned in the hot air oven in all the extractants. The migration of U.V. absorbing materials in the various extracts were in the following order : 5% acetic acid > 5% sodium carbonate > 0.9% sodium chloride > 3% acetic acid > 8% ethanol > distilled water.

Migration of U.V. absorbing material from water tumblers of brand A is shown in Fig. 23. As evident from the figure, migration of U.V. absorbing materials were within the permissible limit when kept in a hot air oven and above the permissible limit under sunlight when distilled water was used as the extractant. The migration of UV absorbing materials were above the permissible limit under both the extracting condition when 8% ethanol, 3% acetic acid, 5% acetic acid, 0.9% sodium chloride and 5% sodium carbonate were used as the extracting media. Under sunlight migration of U.V. absorbing materials were slightly higher in comparison with the migration obtained in the hot air oven kept at similar temperature and duration. The order of migration of U.V. absorbing material in the various extracts was: 5% sodium carbonate > 5% acetic acid > 3% acetic acid > 0.9% sodium chloride > 8% ethanol > distilled water.

The leaching of U.V. absorbing materials from water tumblers of brand B were within the permissible limit under both the conditions of extraction, when distilled water, 8% ethanol and 3% acetic acid were used as the extractants (Fig. 24). However, when extracted with 0.9% sodium chloride, migration of U.V. absorbing materials were above than the permissible limit under sunlight and below the permissible limit at the same temperature maintained in a hot air oven. The migration of U.V. absorbing materials under both the conditions were above than the permissible limit when 5% acetic acid and 5% sodium carbonate were used as the extractants. The degree of increase in U.V. abosrbing materials under sunlight were 15% and 23% in 5% sodium carbonate and 5% acetic acid respectively in comparison with the migration obtained in the hot air oven kept for similar temperature and durations. Migration of U.V. absorbing materials in the various extracts were in the following

order: 5% acetic acid > 5% sodium carbonate > 0.9% sodium chloride > 3% acetic acid > 8% ethanol > distilled water.

The migration of U.V. absorbing materials from lunch boxes of brand A under both the extraction conditions were within the permissible limit when distilled water, 8% ethanol, 3% acetic acid and 0.9% sodium chloride were used as the extractants (Fig. 25). However, when 5% acetic acid and 5% sodium carbonate were used as the extractants migration of U.V. absorbing materials were above than the permissible limit under sunlight and within the permissible limit in the hot air oven maintained at identical temperature and kept for similar durations. It is worthwhile to note that an increased rate of migration of U.V. absorbing materials were noticed under sunlight in all the extracts and in 5% acetic acid and 5% sodium carbonate it was 25% in comparison with the migrations obtained in the hot air oven. The order of migration of U.V. absorbing materials in the various extracting media were:
5% acetic acid > 5% sodium carbonate > 3% acetic acid > 0.9% sodium chloride > 8% ethanol > distilled water.

Migration of U.V. absorbing materials from lunch boxes of brand B is shown in Figure 26. As apparent from the figure, the migration of U.V. absorbing materials were within the permissible limit in all the extracting media when kept in a hot air oven. However, when extracted under sunlight, 3% and 5% acetic acid and 5% sodium carbonate showed migration of U.V. absorbing materials in concentration above than the permissible limit. About 15 to 30% increase in the rate of U.V. absorbing materials were found under sunlight conditions in comparison with the migration obtained in the hot air oven maintained at same temperature. Order of migration of U.V. absorbing materials in various extracts were:

5% sodium carbonate > 5% acetic acid > 3% acetic acid > 0.9% sodium chloride > 8% ethanol > distilled water.

Migration of U.V. absorbing materials from blood bags of brand A is shown in Figure 27. As evident from the figure, migration of U.V. absorbing materials were found to be within the permissible limit in all the extracting media under both the extracting conditions. However, under sunlight an increase rate of migration of U.V. absorbing materials were noticed in all the extracting media in comparison with the migration obtained in the hot air oven kept at identical temperature and durations. Migration of U.V. absorbing materials in various extracts was found to be in the following order:

5% sodium carbonate > 5% acetic acid > 0.9% sodium chloride > 3% acetic acid > 8% ethanol > distilled water.

Migration of U.V. absorbing materials from blood bags of brand B were within the permissible limit in all the extracting media, under both the extracting conditions (Fig. 28). It is interesting to note that under sunlight conditions about 20% to 25% increased rate of U.V. absorbing materials were noticed in comparison with the migrations obtained in the hot air oven kept for similar temperature and duration. Migration of U.V. absorbing materials in various extracts were of the following order:

5% sodium carbonate > 5% acetic acid > 3% acetic acid > 0.9% sodium chloride > 8% ethanol > distilled water.

Influence of sunlight of the global migration from plastic freeze bottles, water tumblers, lunch boxes and blood bags is summarized in Figures 29-36.

Global migration from the freeze bottles of brand A was within the permissible limit when extracted with distilled water, 8%

ethanol and 3% acetic acid under sunlight and at the same temperature (40-45°C) in a hot air oven (Fig-20). However, when extracted with 5% acetic acid and 0.9% sodium chloride, global migration was above than the permissible limit under sunlight and within the permissible limit in the hot air oven. It is interesting to note that when extracted with 5% sodium carbonate, global migration above than the permissible limit was noticed under both the conditions of extraction. Maximum global migration was noticed in 5% sodium carbonate and the order of migration in various extracts was:

5% sodium carbonate > 5% acetic acid > 0.9% sodium chloride > 3% acetic acid > 8% ethanol > distilled water.

Influence of sunlight on the gobal migration from freeze bottles of brand B is shown in Figure 30. It is evident from the figure, global migration was within the permissible limit when distilled water, 8% ethanol, 3% acetic acid and 0.9% sodium chloride were used as the extractants under both the extracting conditions. However, when 5% acetic acid and 5% sodium carbonate were used as the extractants, global migration was found to be above than the permissible limit, under both the extracting conditions. Maximum migration was noticed in 5% sodium carboante and the order of leaching in various extracts was: 5% sodium carbonate > 5% acetic acid > 3% acetic acid > 0.9% sodium chloride > 8% ethanol > distilled water.

It is apparent from the Figure 31 that from water tumblers of brand A global migration was found to be within the permissible limit, when extracted with distilled water, 8% ethanol and 3% acetic acid under both the test conditions. While in 5% sodium carbonate, 5% acetic acid and 0.9% sodium chloride the global migration was above than the permissible limit under both the extracting conditions. The

order of global migration in various extractants was:

5% sodium carbonate > 0.9% sodium chloride > 5% acetic acid > 3% acetic acid > 8% ethanol > distilled water.

Figure 32 shows influence of sunlight on the global migration from water tumblers of brand B. As apparent from the figure, the global migration was within the permissible limit in distilled water and 8% ethanol under both the extracting conditions. However, when 3% and 5% acetic acid, 0.9% sodium chloride and 5% sodium carbonate were used as the extractants the global migration was above than the permissible limit under both the extracting conditions. It is interesting to note that water tumblers of brand B showed maximum migrations in acetic media while, in other samples maximum migration was noticed in basic medium. The degree of global migrations obtained under the sunlight was slightly higher in comparison with the migrations obtained in the hot air oven maintained at the same temperature. The order of global migrations in various extracts was found to be:

5% acetic acid > 5% sodium carbonate > 3% acetic acid > 0.9% sodium chloride > 8% ethanol > distilled water.

Influence of sunlight on the global migration from lunch boxes of brand A is shown in Figure 33. As evident from the figure, the global migration was within the permissible limit, in all the extracting media under both the extracting conditions, except 5% sodium carbonate, where the global migration was above than the permissible limit under both the extracting conditions. It is of significance to note that under sunlight the degree of global migration obtained was slightly higher in comparison with the migrations obtained in hot air oven in all the extracts. The order of global migration in various extracts was:

5% sodium carbonate > 5% acetic acid > 3% acetic acid > 0.9% sodium chloride > 8% ethanol > distilled water.

Effect of sunlight on the global migration from lunch boxes of brand B is shown in Figure 34. As evident from the figure, the global migration was within the permissible limit, when distilled water and 8% ethanol were used as the extractants under both the extracting conditions. On the other hand, when extracted with 3% and 5% acetic acid, 5% sodium carbonate and 0.9% sodium chloride, the global migration was found to be above than the permissible limit under both the extracting conditions. It is interesting to note that under sunlight an increased rate of global migration was noticed in all the extracts except 8% ethanol, with the migrations obtained in the hot air oven maintained at the identical temperature. The order of migration in various extracts was:
5% sodium carbonate > 5% acetic acid > 3% acetic acid > 0.9% sodium chloride > 8% ethanol > distilled water.

Influence of sunlight on the global migration from blood bags of brands A and B are shown in Figure 35 and 36 respectively. As evident from the figures, the global migration obtained from both the brands of blood bags was found to be within the permissible limit in all the extracting media under both the extracting conditions. However, from blood bags of brand A, the global migration in 8% ethanol was higher than that of in 3% acetic acid and the migration obtained in 0.9% sodium chloride was higher than that of in 3% acetic acid. On the other hand, in other samples studied, the migrations obtained in 3% acetic acid and 5% acetic acid was comparatively higher than that of in 8% ethanol and 0.9% sodium chloride respectively. The order of global migration in various extracts from both the brands of blood bags was:

5% sodium carbonate > 0.9% sodium chloride > 5% acetic acid > 8% ethanol > 3% acetic acid > distilled water.

Influence of pH and temperature on the migration of heavy metals from plastic freeze bottles, water tumblers, lunch boxes and blood bags is shown in Tables 22-28.

As evident from Table 22, migration of heavy metals from freeze bottles of brand A was within the permissible limit, in distilled water at all the extraction temperatures (40°C for 8 hours, 24 hours and 60°C for 2 hours, and 240 hours). However, when 3% and 5% acetic acid and 5% sodium carbonate was used as the extractants, leaching of Cd occurred in amounts above than the permissible limit at all the extracting temperatures, except at 40°C for 8 hours where leaching of metals were within the permissible limit. It is interesting to note that when 5% acetic acid (pH = 2.5) and 5% sodium carbonate (pH=10) were used as the extracting media. There was significant increase in the migration of Cd in comparison to the migration obtained in 3% acetic acid.

Table 23, summarizes the results of the extraction studies with freeze bottles of brand B. It is apparent from table, leaching of Pb and Zn occurred in concentrations above than the permissible limit when extracted with distilled water at 60°C for 240 hours, while none of the metals leached out in amounts above than the permissible limit at other extracting temperatures. When extracted with 3% acetic acid, in addition to the leaching of Pb and Zn at 60°C for 240 hours, leaching of Zn also occurred in concentrations above than the permissible limit at 40°C for 24 hours which was not noticed in distilled water. It is interesting to note that the increased rate of Pb was found to be significant in 3% acetic acid with respect to distilled water at 60°C for

240 hours. During the extraction studies with 5% acetic acid, in addition to the migration of Pb and Zn at above temperature leaching of Zn also occurred above than the permissible limit at 60°C for 2 hours. The increased rate of Pb and Zn in 5% acetic acid (PH = 2.5) at 60°C for 240 hours was also significant with respect to 3% acetic acid (pH=4.0). When extracted with 5% sodium carbonate, leaching of Pb occurred in amounts above than the permissible limit at all the extracting temperatures except at 40°C for 8 hours, where it was within the permissible limit and Cd also appeared at 60°C for 240 hours.

Table 24 shows the results of the extraction studies with water tumblers of brand A. As evident from the table, migration of Cd in concentrations above than the permissible limit were found at all the extracting temperatures and at 60°C for 240 hours in addition to Cd, Cu also leached out in distilled water. When extracted with 3% and 5% acetic acid and 5% sodium carbonate same metals leached out in all the extracts at all the extracting temperatures, with the exceptions that leaching Cr also occurred in all the extracts at 40°C for 24 hours and 60°C for 240 hours. However, concentrations of metals leached out in these extracts were found to be significantly higher at most of the extracting temperature than the obtained in distilled water. It is interesting to note that in 5% acetic acid the increased rate of leaching of Cd, was found to be also significant with respect to 3% acetic acid at 40°C for 8 hours and 24 hours and at 60°C for 2 hours, and that of Cr only at 40°C for 24 hours and Cu at 60°C for 240 hours.

As evident from the Table 25, none of the metals leached out in amounts above than the permissible limit from water tumblers of brand B in distilled water under all the extracting temperatures.

However, when the extraction studies were carried out with 3% and 5% acetic acid and 5% sodium carbonate leaching of Cd occurred in concentrations above than the permissible limit in all extracts under all the extracting temperatures except at 40°C for 8 hours where it was below the permissible limit. It is worthwhile to note that the leaching of Cd in 5% acetic acid (pH = 2.5) and 5% sodium carbonate (pH = 10) was significantly higher in comparison with the migration obtained in 3% acetic acid (pH = 4.0).

It is apparent from Table 26 , lunch boxes of brand A showed migration of Cd and Pb in amounts above than the permissible limit at 40°C for 8 hours, while, at 60°C for 2 hours leaching of Cd and Cu occurred and at 60°C for 240 hours including Cd and Cu, leaching of Pb also occurred in concentrations above than the permissible limit when distilled water was used as the extractants. It is interesting to note that same metals leached out when the extraction studies were carried out with 3% and 5% acetic acid. This sample also showed an increased rate of Cd in 3% acetic acid at all the extracting temperatures with respect to distilled water. While in 5% acetic acid at 60°C for 240 hours the increased rate of Cu and Cd were also significant with respect to 3% acetic acid. During the extraction studies with 5% sodium carbonate leaching of only Cd occurred in concentrations above than the permissible limit at all the extracting temperature and at 60°C for 240 hours including Cd, leaching of Pb also occurred above than the permissible limit. The significant increased rate of Cd at 40°C for 8 hours was found to be significant with respect to distilled water and at 60°C for 240 hours the increased rate was also significant with respect to 3% acetic acid.

Table 27 summarizes the result of extraction studies with lunch boxes of brand B. As evident from the table, leaching of Cd and Zn in amounts above than the permissible limit was found at all the extracting temperature except at 40°C for 8 hours where none of the metals leached out above than the permissible limit. When the extraction studies were carried out in 5% sodium carbonate, same metals leached out in all the extracting temperatures with the exceptions that leaching of Cd also occurred at 40°C for 8 hours which was not found in distilled water. During the extraction studies with 3% and 5% acetic acid same metals migrated at 40°C for 8 hours as seen in 5% sodium carbonate. However, when extracted for 24 hours at 40°C and at 60°C for 10 days including Cd and Zn, leaching of Cr also occurred in amounts above than the permissible limit. However, migration of metals in 5% acetic acid was slightly higher than that obtained in 3% acetic acid under all the extracting temperatures.

Results of extraction studies with blood bags of brand A is shown in Table 28. As evident from table, the migration of all the metals within the permissible limit was found under all the extracting temperature, except migration of Pb at 60°C for 10 days in distilled water. When the extraction studies were carried out with 3% acetic acid, leaching of Pb occurred in amounts above than the permissible limit at 40°C for 24 hours and at 60°C for 2 hours, while at 60°C for 240 hours including Pb, leaching of Zn also occurred in concentrations above than the permissible limit and none of the metals leached out at 40°C for 8 hours. More or less a similar pattern of migration of metals were noticed in 5% acetic acid with only the exceptions that leaching of Pb also occurred at 40°C for 8 hours which was not detected

in 3% acetic acid. Concentrations of Pb leached out in 5% acetic acid was significantly higher than that obtained in distilled water at 65°C for 240 hours. When extracted with 5% sodium carbonate pattern of leaching of metals were same as that observed in 3% acetic acid, with the exceptions that leaching of Pb at 60°C for 240 hours was significantly higher than that obtained in distilled water which was not significantly increased in 3% acetic acid.

Influence of pH and temperature were also examined in one more brand of blood bags. It is of importance to note that none of the metals leached out in concentrations above than the permissible limit under any extracting temperature and extracting media used (data not shown).

Effect of pH and temperature on the migration of U.V. absorbing materials from plastic freeze bottles, water tumblers, lunch boxes and blood bags is summarised in Tables 29-36

As apparent from Table 29 freeze bottles of brand A showed the migration of U.V. absorbing materials below the permissible limit at all the extracting temperatures except at 60°C for 240 hours where they were above than the permissible limit, in distilled water. However, when extracted with 3% acetic acid the migration of U.V. absorbing materials were above than the permissible limit at all the extracting temperatures, except at 40°C for 8 hours where they were within the permissible limit. When 5% acetic acid and 5% sodium carbonate were used as the extractants, the migration of U.V. absorbing materials were above than the permissible limit at all the extracting temperatures.

As shown in Table 30 that the migration of U.V. absorbing materials from freeze bottles of brand B were within the permissible

limit at all the extracting temperatures when distilled water and 3% acetic acid were used as the extractants. When extracted with 5% acetic acid and 5% sodium carbonate the migration of U.V. absorbing materials above than the permissible limit were noticed at 60°C for 240 hours and at other extracting temperatures they were within the permissible limit. The order of leaching of U.V. absorbing materials in different extractants was:

5% sodium carbonate > 5% acetic acid > 3% acetic acid > distilled water.

As evident from Table 31 that the leaching of U.V. absorbing materials were found to be above the permissible limit in all the extracts at all the extracting temperatures, except at 40°C for 8 hours in distilled water, where it was within the permissible limit. The order of migration of U.V. absorbing materials in the various extracts was:

5% sodium carbonate > 5% acetic acid > 3% acetic acid > distilled water.

As evident from Table 32, water tumblers of brand B showed migration of the U.V. abosrbing materials within the permissible limit at all the extracting temperatures when distilled water was used as the extractant. However, when extracted with 3% and 5% acetic acid and 5% sodium carbonate the U.V. absorbing materials migrated in amounts above the permissible limit at all the extracting temperatures, except at 40°C for 8 hours in 3% acetic aid where they were within the permissible limit. The order of migration in various extracts was:

5% acetic acid > 3% acetic acid > 5% sodium carbonate > distilled water.

Table 33 summarizes the results of the extraction studies with lunch boxes of brand A. It is shown in the table that migration of U.V. absorbing materials within the permissible limit were found

at all the extracting temperatures except at 60°C for 240 hours where they were above than the permissible limit when distilled water and 3% acetic acid were used as the extracting media. However, when extracted with 5% acetic acid and 5% sodium carbonate, the migration of U.V. absorbing materials occurred in concentrations above than the permissible limit also at 40°C for 24 hours. This sample also showed maximum leaching of U.V. absorbing materials in acidic medium and the order of migration in various extracts was:

5% acetic acid > 3% acetic acid > 5% sodium carbonate > distilled water.

It is shown in Table 34, that lunch boxes of brand B showed migration of U.V. absorbing materials within the permissible limit at all the extracting temperatures in distilled water. However, when extracted with 3% and 5% acetic acid and 5% sodium carbonate the migration of U.V. absorbing materials occurred in amounts above than the permissible limit at all the extracting temperatures. The order of leaching of U.V. absorbing material in various extracts was:

5% sodium carbonate > 5% acetic acid > 3% acetic acid > distilled water.

It is apparent from Table 35, that the migration of U.V. absorbing materials from blood bags of brand A were found to be within the permissible limit at all the extracting temperatures in distilled water and 3% acetic acid. However, when the concentration of acetic acid was increased from 3% (pH = 4.0) to 5% (pH = 2.5), the U.V. absorbing materials were found to migrate out in amounts above than the permissible limit at 60°C for 240 hours. When extracted with 5% sodium carbonate the migration of U.V. absorbing materials occurred in amounts above than the permissible limit at all the extracting temperatures except at 40°C for 8 hours, where they were within the permissible limit. The order of migration of U.V. absorbing materials in various extracts was:

5% sodium carbonate > 5% acetic acid > 3% acetic acid > distilled water.

It is evident from Table 36 that the migration of U.V. absorbing materials from blood bags of brand B were found to be within the permissible limit at all the extracting temperatures in all the extractants. The migration of U.V. absorbing materials in various extracts was in the following order:

5% sodium carbonate > 5% acetic acid > 3% acetic acid > distilled water.

Influence of pH and temperature on the global migration from plastic freeze bottles, water tumblers, lunch boxes and blood bags is shown in Tables 37-44.

As evident from Table 37, freeze bottles of brand A showed global migration within the permissible limit at all the extracting temperatures when distilled water was used as the extracting medium. However, an increased rate of global migration was noticed with the increase of extraction period, keeping the temperature constant. When extracted with 3% acetic acid the global migration was again found to be within the permissible limit at all the extracting temperatures except at 60°C for 240 hours where it was above than the permissible limit. It is of importance to note that the increased rate of global migration was found to be significant at all the extracting temperatures with respect to distilled water. When extracted with 5% acetic acid, the global migration was found to be above than the permissible limit at all the extracting temperatures except at 40°C for 8 hours, where it was below the permissible limit. The increased rate of global migration in 5% acetic acid was also found to be significant with respect to 3% acetic acid at all the extracting temperatures. When 5% sodium carbonate was used as the extractant the global migration was found to be above than the

permissible limit, with significant increase at all the extracting temperatures, which was not found in other extracting media.

As evident from Table 38, the global migration from freeze bottles of brand B was within the permissible limit at all the extracting temperatures, except at 60°C for 240 hours where it was above than the permissible limit in distilled water. About 50% increase in the global migration was observed with the increase of the durations of extraction from 2 hours to 240 hours. Keeping the temperature (60°C) constant. When extracted with 3% acetic acid the global migration was above than the permissible limit at 40°C for 24 hours and at 60°C for 240 hours, while at 40°C for 8 hours and 60°C for 2 hours, it was within the permissible limit. However, an increased rate of global migration was found at all the extracting temperatures with respect to distilled water. When 5% acetic acid and 5% sodium carboante were used as the extractants the migration was above than the permissible limit at all the extracting temperatures and the increased rate of global migration was also found to be significant with respect to 3% acetic acid at all the extracting temperatures, except at 60°C for 2 hours in 5% acetic acid where the increased rate was significant only with respect to distilled water. In both the extracts the increase in the duration of extraction from 2 hours to 240 hours at 60°C and 8 hours and 24 hours at 40°C resulted more than 50% increase of global migration.

As apparent from Table 39, water tumblers of brand A showed global migration within the permissible limit at 40°C for 8 hours and 24 hours in distilled water. However, at 60°C for 2 hours and 240 hours, it was above than the permissible limit. When 3% acetic acid was used as the extractant, the global migration was found to be above than

the permissible limit at 60°C for 2 hours it was within the permissible limit. The increased rate of global migration was found to be significant at 40°C for 8 hours and at 60°C for 240 hours with respect to distilled water. It is interesting to note that in 5% acetic acid and 5% sodium carbonate the global migration was found to be above than the permissible limit with an increased rate of global migration which was found to be significant with respect to 3% acetic acid also, at all the extracting temperatures. With the increase in duration of extracting, keeping the temperature constant, about 50% increase in the level of global migration was noticed.

As apparent from Table 40, water tumblers of brand B showed global migration within the permissible limit at all the extracting temperatures except at 60°C for 240 hours, where it was above than the permissible limit in distilled water. It is interesting to note that in 3% and 5% acetic acid and 5% sodium carbonate the global migration was found tobe above than the permissible limit at all the extracting tempera-
noticed in acidic medium,while in other samples the max. migration was
tures. In this sample the maximum migration was found in alkaline medium.

Table 41 shows the results of the extraction studies with lunch boxes of brand A. As evident from the table, globalmigration was above than the permissible limit only at 60°C for 240 hours while at other extracting temperatures it was within the permissible limit during the extraction with distilled water. When extracted with 3% acetic acid the global migrations above than the permissible limit was also observed at 40°C for 24 hours. However, the increased rate of global migration was significant at all the extracting temperatures with respect to distilled water. Similar observations were made with 5% acetic acid but the increased rate of global migration was also significant with

respect to 3% acetic acid at all the extracting temperatures. It is interesting to note that in 5% sodium carbonate the global migration was found to be above than the permissible limit and the increased rate was significant with respect to 3% acetic acid at all the extracting temperatures.

As evident from Table 42, the lunch boxes of brand B showed global migrations within the permissible limit at all the extracting temperatures, except at 60°C for 240 hours where it was above than the permissible limit when extracted with distilled water. In 3% acetic acid, the global migration was found to be above than the permissible limit at all the extracting temperature. The increased rate of global migration was also found to be significant with respect to 3% acetic acid at all the extracting temperature, except at 40°C for 8 hours in 5% acetic acid, where the increased rate was significant only in comparison with distilled water.

Table 43 summarizes the results of the extraction studies with the blood bags of brand A. As evident from the table, global migration was within the permissible limit at all the extracting temperatures in distilled water and 3% acetic acid. However, in 3% acetic acid an increased rate of global migration was observed which was significant with respect to distilled water at all the extracting temperatures. When extracted with 5% acetic acid and 5% sodium carbonate the global migration was found to be within the permissible limit at all the extracting temperatures, except at 60°C for 240 hours, where it was above than the permissible limit. It is interesting to note that the increased rate of global migration was found to be significant in comparison with 3% acetic acid at all the extracting temperature in 5% acetic acid and 5% sodium carbonate.

Blood bags of brand B showed global migrations within the permissible limit in all the extracts at all extracting temperature (Table 44). However, in 3% acetic acid global migration was found to be significantly higher in comparison to distilled water at all the extracting temperatures. In 5% acetic acid and 5% sodium carbonate the increased rate was also significant with respect to 3% acetic acid at all the extracting temperatures.

DISCUSSION:

The results demonstrated that the migration of chemical additives (viz. U.V. absorbing materials, heavy metals etc.) increased with the increase in the acidity and alkalinity of the extracting media. It also increased with the increase in temperature and time of extraction. It was also observed that sunlight accentuates the migration of U.V. absorbing materials and heavy metals. The influence of sunlight on the global migration appears insignificant in our experimental conditions but, however, it assumes paramount significance as the exposure of plastics are generally perennial, in which case it might enhance the leachability of the chemical additives. It was interesting to note that under sunlight, selective migration of Mn occurred from some of the plastic samples which was not detected in the hot air oven maintained at the same temperature and duration and even after increasing the temperature and time of extraction. It is difficult to pin point the exact factor responsible for such an increased rate of migration of U.V. absorbing materials and heavy metals under sunlight. Possibly, factors such as some radiations like U.V. and oxidants like ozone and some other rays present in the environment of sunlight exposure could be responsible which are not otherwise found in the hot air oven. Polymer fragmentation

under sunlight and specially under U.V. rays has been reported (184).

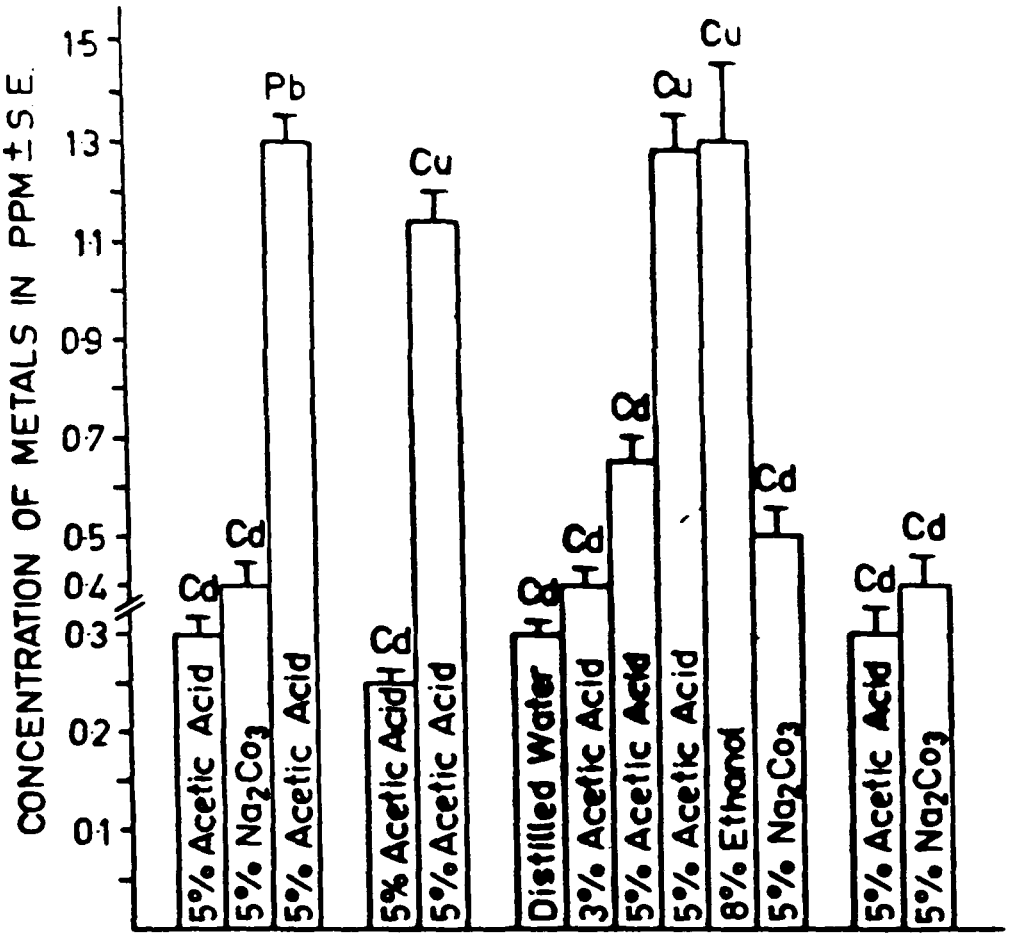
Out of eight brands of plastic samples studied, six samples showed maximum global (overall) migration in basic medium and two samples in acidic medium in comparison to the global migration obtained in aqueous, alcoholic and saline media. This suggests that migration of chemical additives are increased with the increase of acidity and basicity, which could be due to the polarization of bonds of the various additives attached with the polymer in the extracts having acidic and basic pH.

Significant concentrations of some of the leachable chemical additives of plastics have been detected in the environment and tissues of animals and human beings. The levels of injurious chemicals in the human may further be increased as a result of their leaching from the finished plastic products.

The present results have suggested that the use of plastic articles may be hazardous if used by the consumers for purposes other than those for which they have been tested our studies have shown that various physico-chemical factors such as sunlight, higher temperature, acidic and basic pH enhanced the migration of injurious chemical additives from finished plastics. Use of plastic utensils or pouches for the storage and packaging of food, drinking water and biological fluids for longer duration is also not advisable as the migration of chemical additives increased significantly with the increase of the time of extraction. It is also important to underscore at this juncture that the various brands of plastic materials studied, when used for shorter durationof time (upto 24 hours) at room temperature (25°C) will not pose a serious threat to the consumers, irrespective of the quality or nature of the food stored, since migration of chemical additives under such conditions

were within the permissible limit. On the basic of these observations it may be concluded that the possibility of the health hazards to the consumers of plastic exist, if non-food grade or untested plastics are used, since some of the additives have toxicogenic potential. Thus the present studies indicate that migration of toxic compounds from the plastics into the food items and stored biological fluids may pose health risks if the safety precautions are not made during manufacture and storage. Our observations are of immense significance as they could serve as a baseline data for formulating the guidelines for the safe use of plastics.

Fig 5 Effect of storage time on the migration of heavy metals (APL) from plastic freeze bottles and water tumblers of brands A & B at 25°c



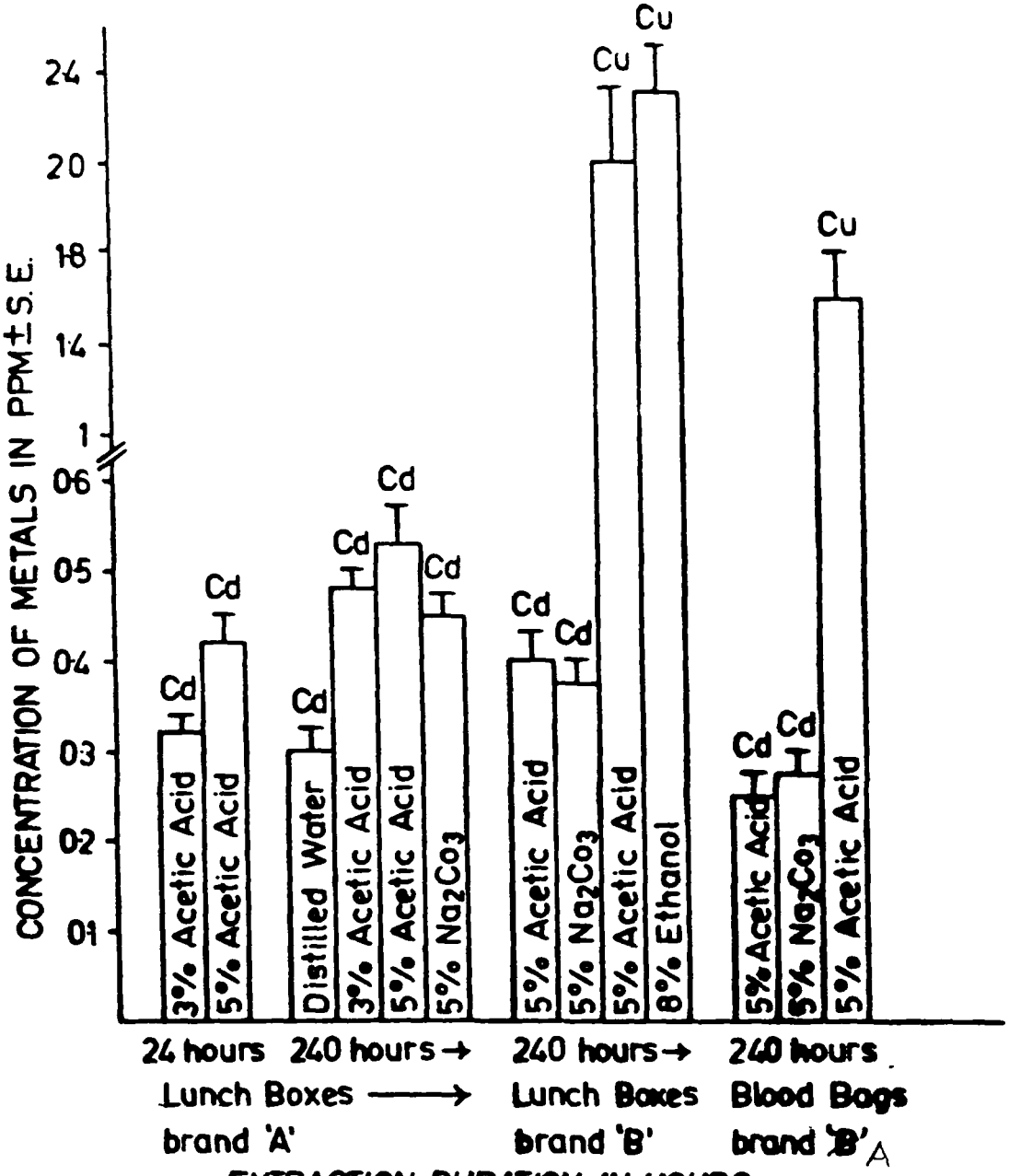
: APL – Above than the permissible limits.

: None of the metals leached out in concentrations above than the permissible limit when extracted for 2 hours and 8 hours.

: The extractants not showed in the figure did not contribute leaching of any metals in amounts above than the permissible limit at that conditions.

: Values are mean ± S.E of four samples, $P < 0.05$ was considered to be significant, (Student's 't' test).

Fig 6 Effect of storage time on the migration of heavy metals (APL) from plastic lunch boxes and blood bags of brands A & B at 25°c



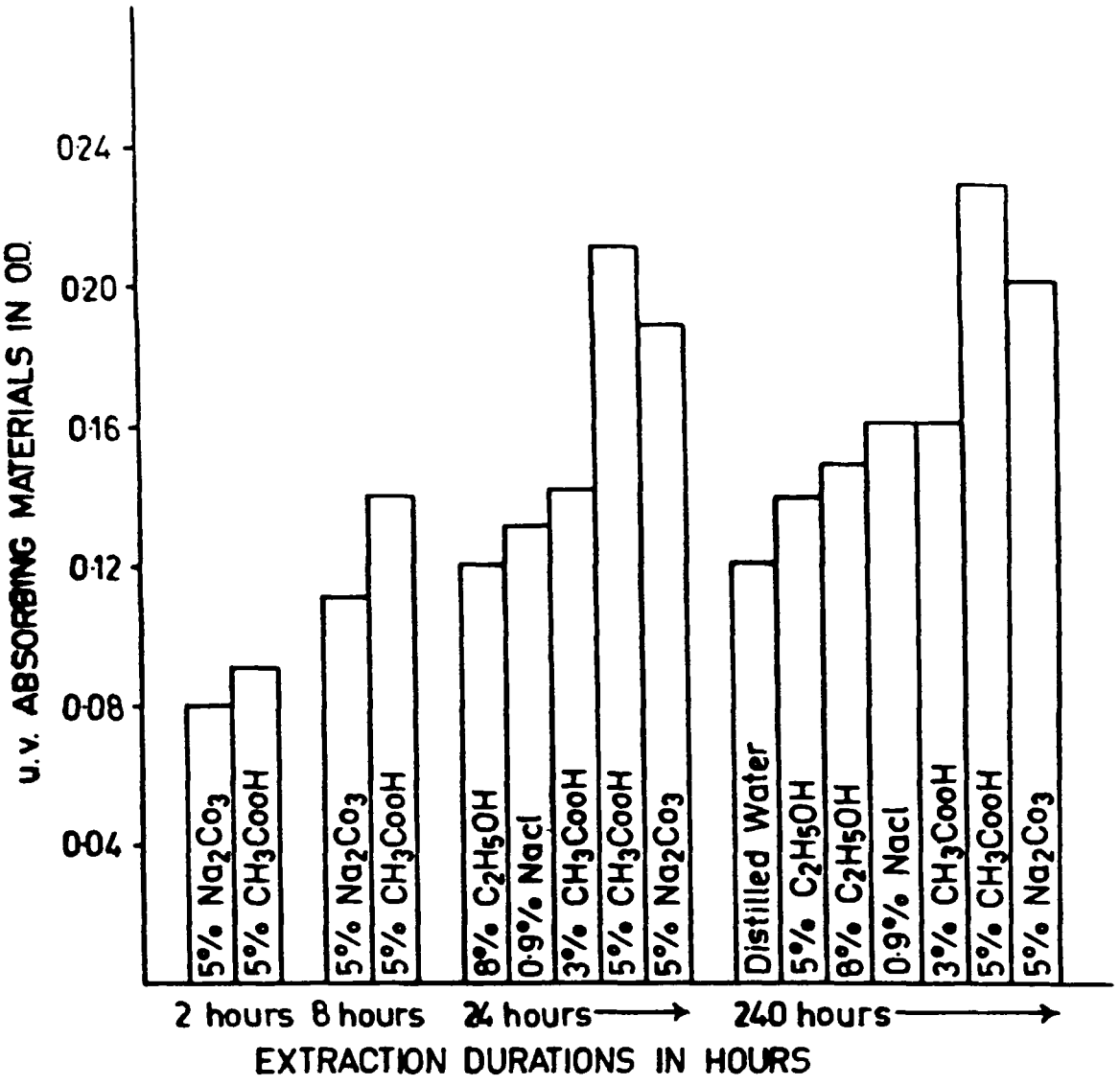
: APL–Above than the permissible limits.

: None of the metals leached out in concentrations above than the permissible limits when extracted for 2 hours and 8 hours.

: The extractants not showed in the figure did not contribute leaching of any metals in amounts above than the permissible limits at that conditions.

: Value are mean ±S.E. of four samples, $P < 0.05$ was considered to be significant, (Student's 't' test).

Fig 7. Influence of storage time on the migration of u.v. absorbing materials in OD from freeze bottles of brand A at 25°c



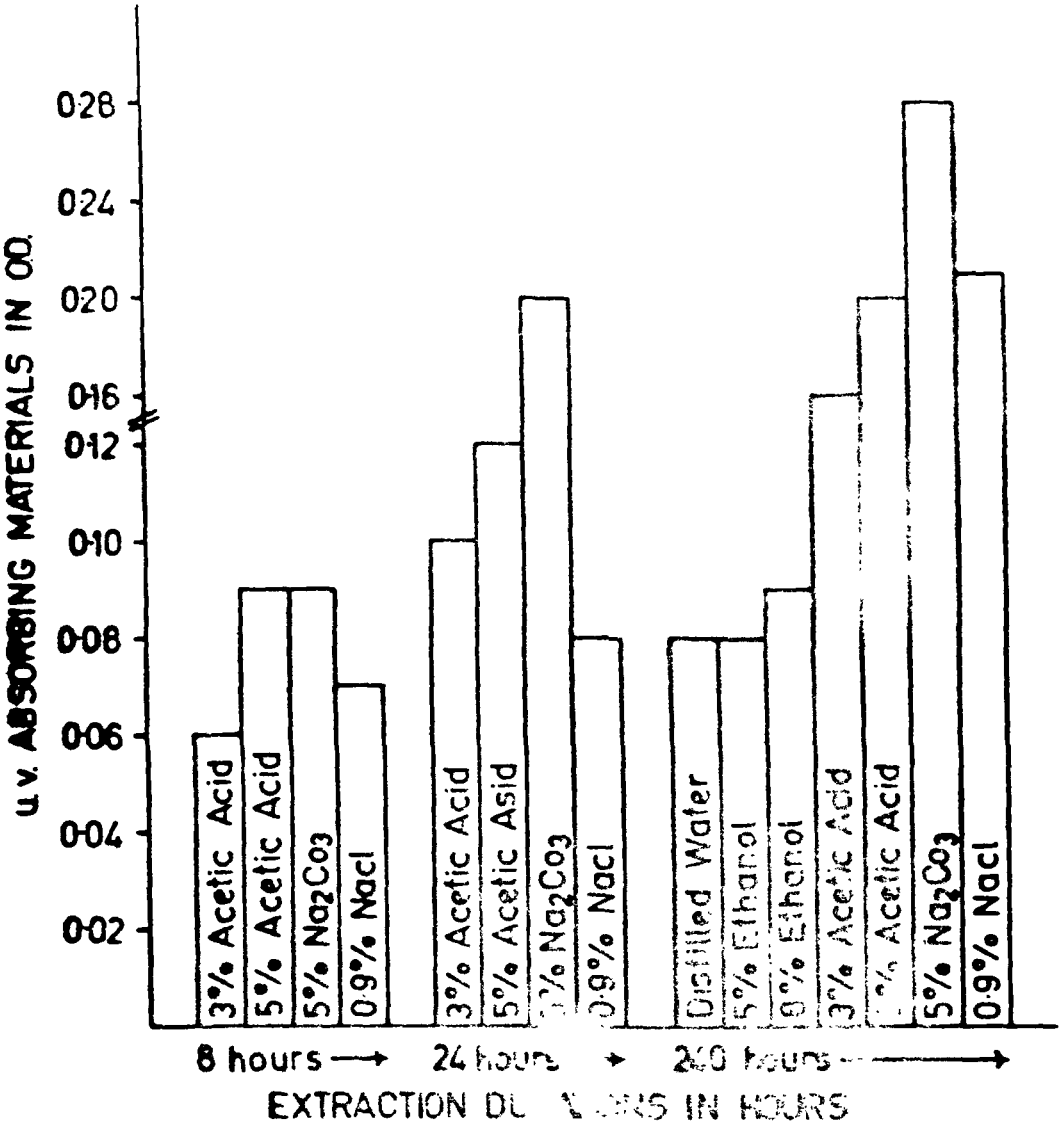
* : Above than the permissible limit. (PL)

: The extractants not showed in the figure did not contribute leaching of u.v. absorbing materials in detectable amounts.

: Values are the average of four samples.

PL : Should not exceed 0·3 O.D.

Fig 8 Influence of storage time on the migration of u v absorbing materials in OD from freeze bottles of B brand at 25°c



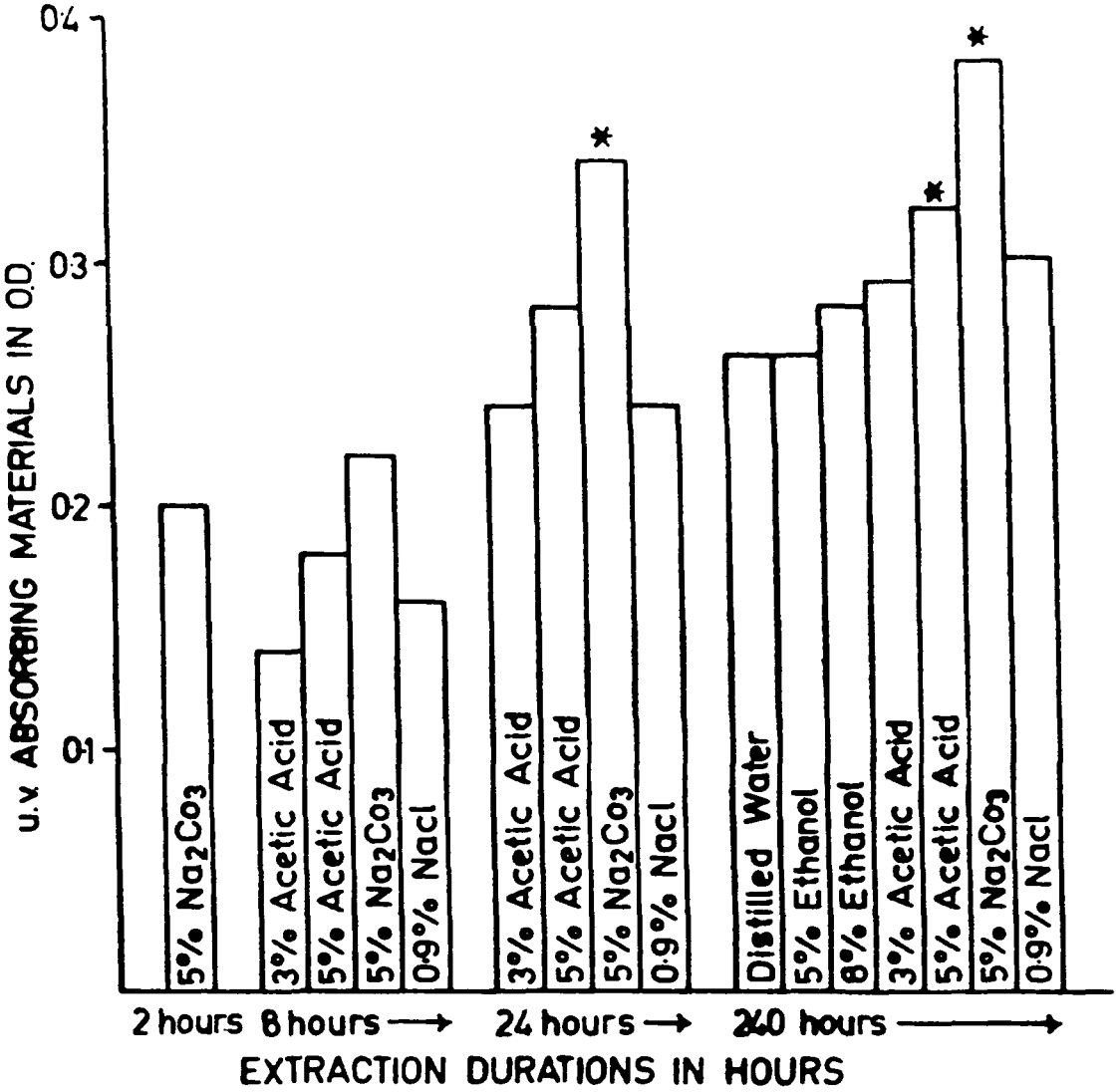
: Leaching of u.v absorbing materials at 25°c kept for 2 hour were found to be nill in all the extracts.

: The extractants not showed in the figure did nc. contribute leaching of u.v. absorbing materials in detectable amounts.

: Values are the average of four samples.

PL : Should not exceed 0·3 O.D.

Fig 9. Influence of storage time on the migration of u v absorbing materials in OD. from water tumblers of A brand at 25°c



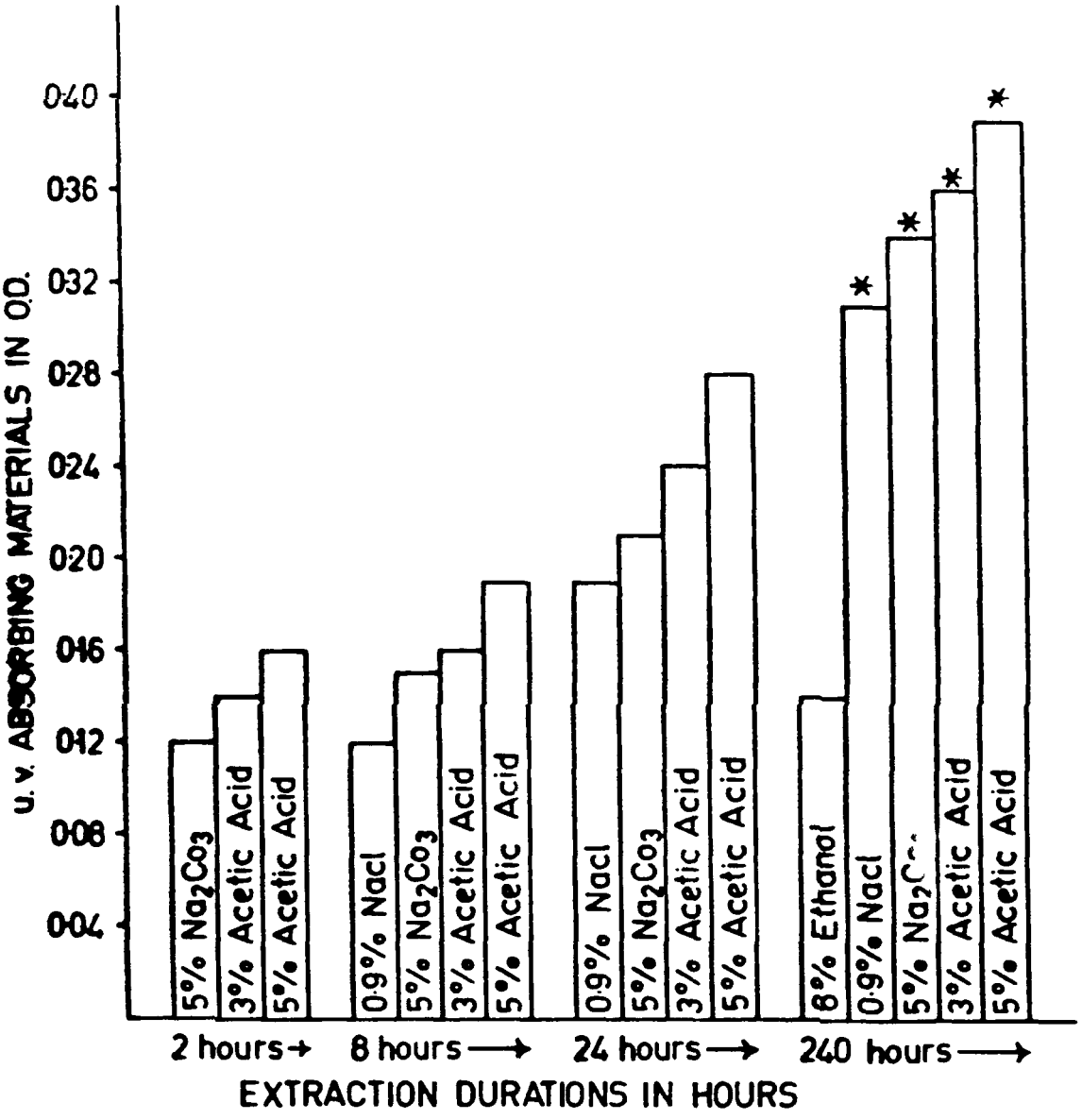
* : Above than the permissible limit. (PL)

: The extractants not showed in the figure did not contribute leaching of u.v absorbing materials in detectable amounts.

: Values are the average of four samples.

PL : Concentration of u.v. absorbing materials should not exceed 0·3 O.D.

Fig 10 Influence of storage time on the migration of u v absorbing materials in OD from water tumblers of B brand at 25°c



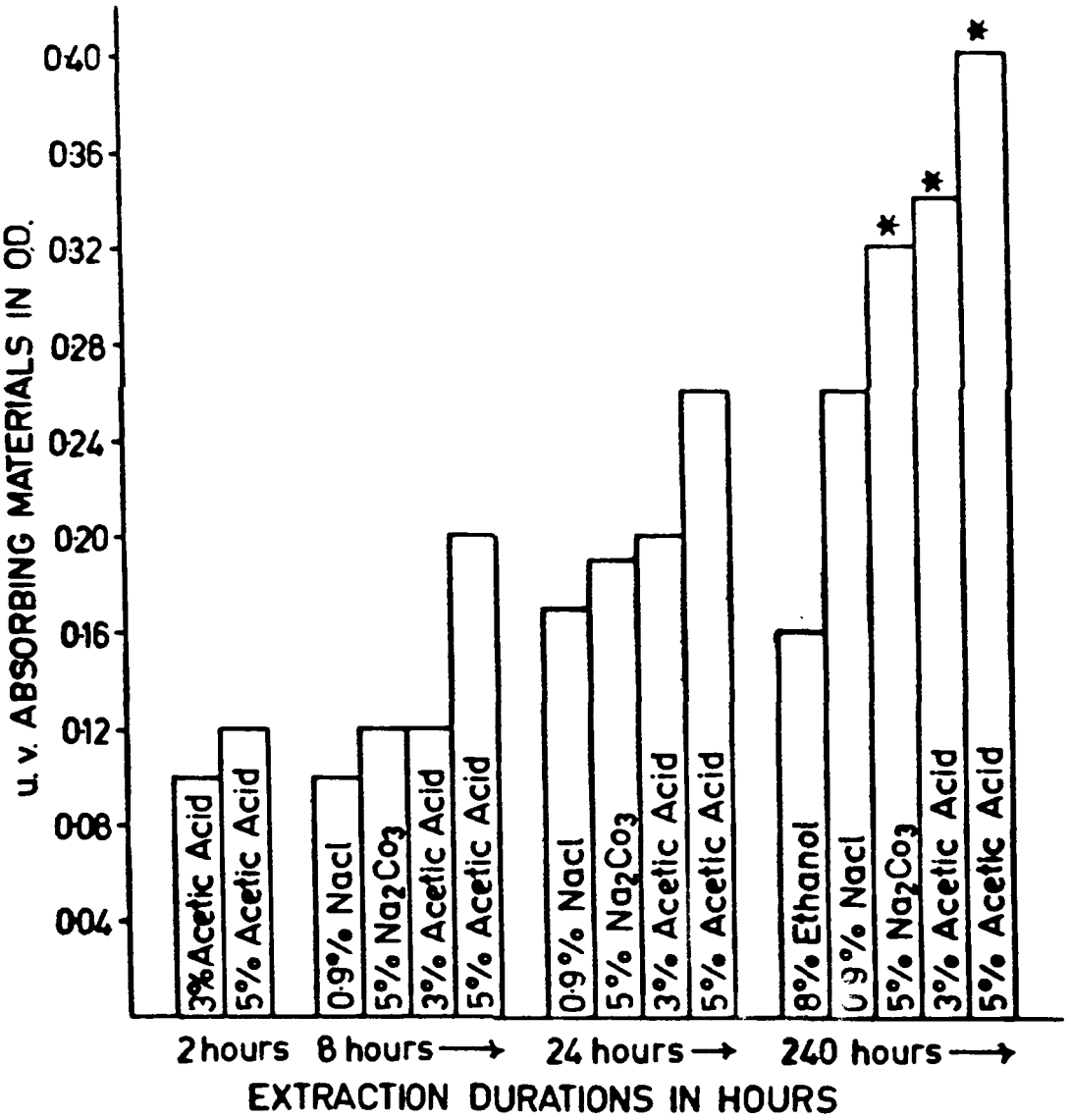
* : Above than the permissible limit. (PL)

: The extractants not showed in the figure did not contribute leaching of u.v. absorbing materials in detectable amounts.

: Values are the average of four samples.

PL : Concentrations of u.v. absorbing materials should not exceed 0·3 O.D.

Fig: 11. Influence of storage time on the migration of u.v absorbing materials in OD from lunch boxes of A brand at 25°c.



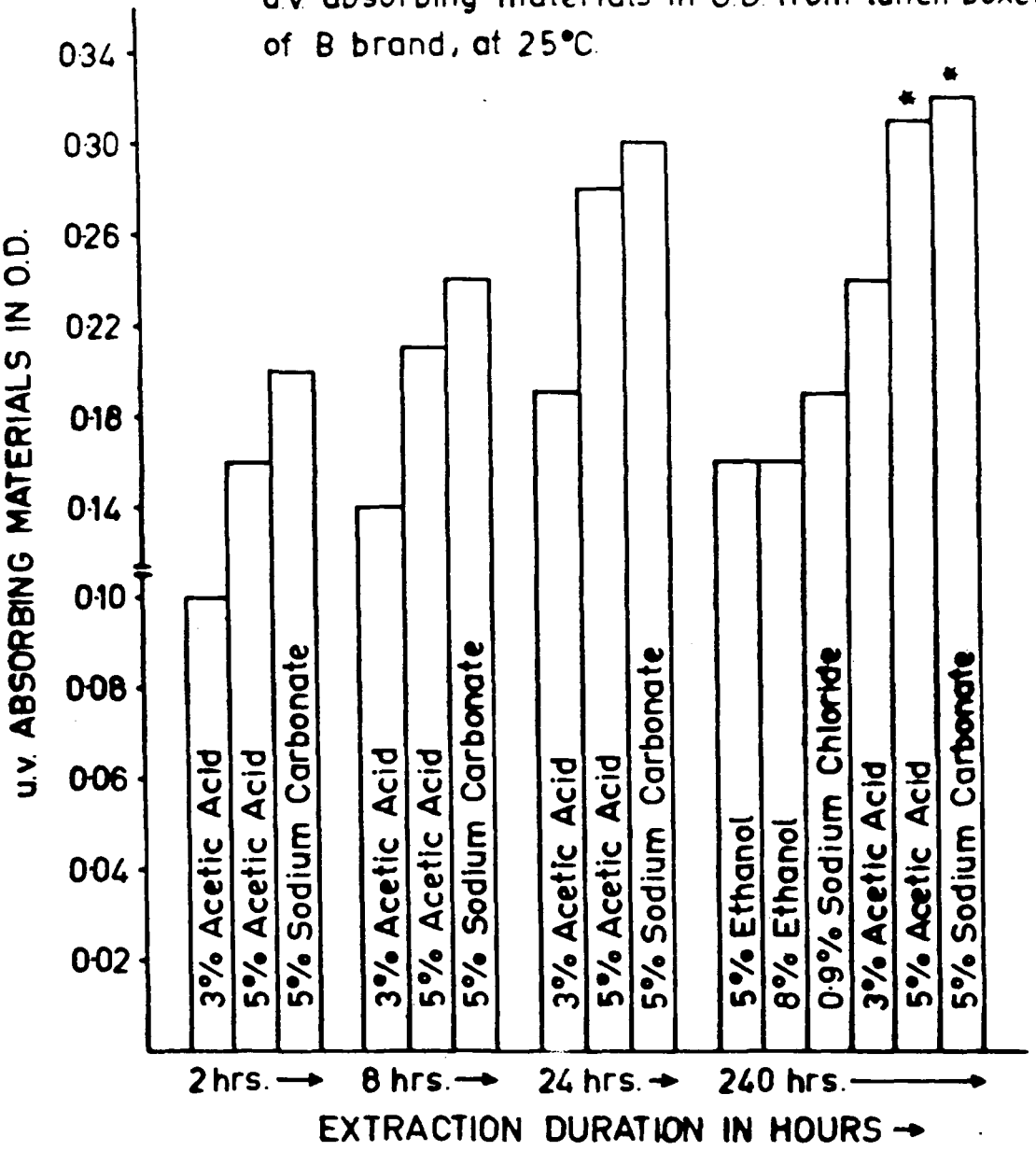
* : Above than the permissible limit. (PL)

: The extractants not showed in the figure did not contribute leaching of u.v. absorbing materials in detectable amounts.

: Values are the mean average of four samples.

PL : Concentrations of u.v. absorbing materials should not exceed 0·3 O.D.

Fig: 12 Influence of storage time on the migration of u.v. absorbing materials in O.D. from lunch boxes of B brand, at 25°C.



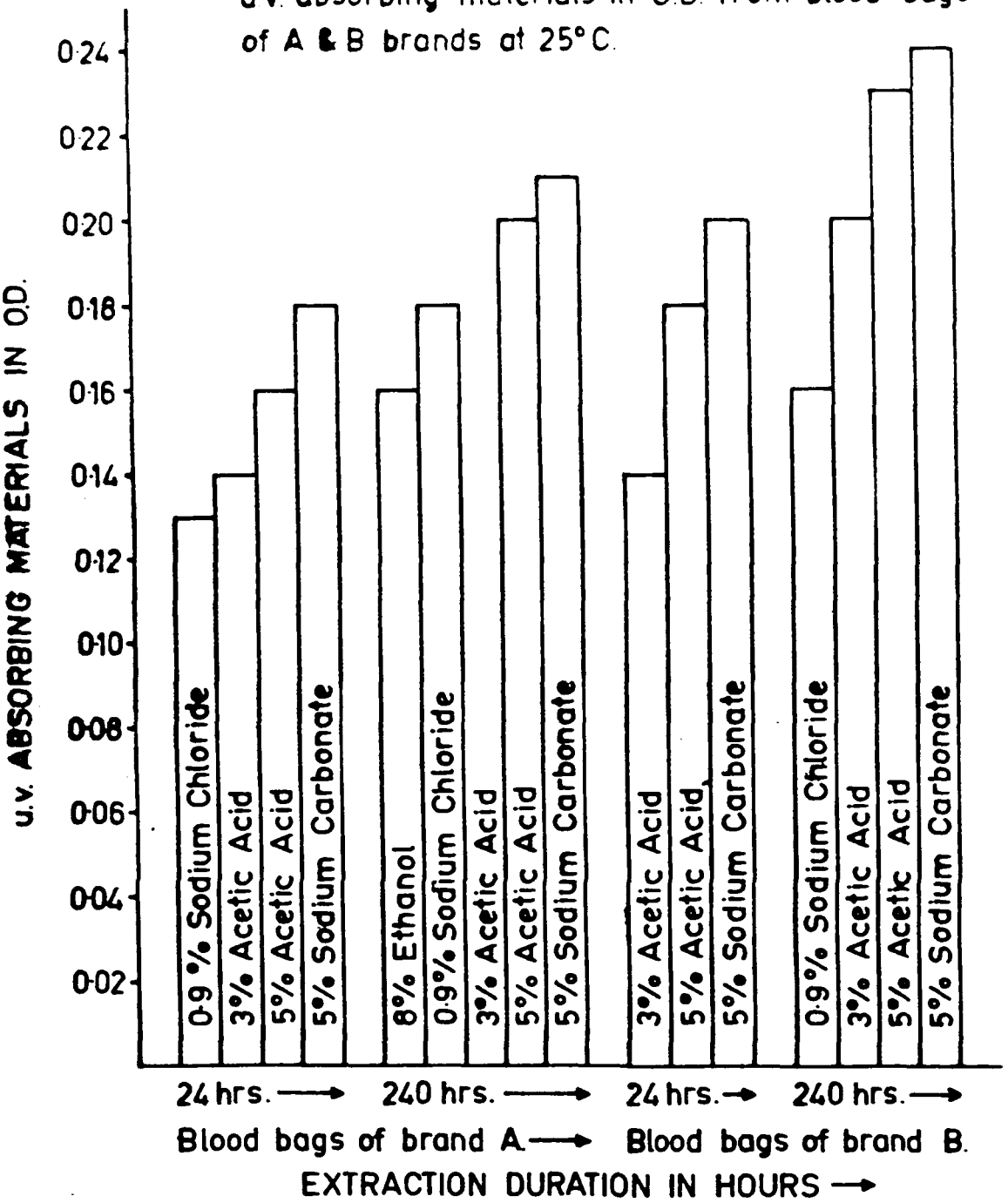
Permissible limit (PL) : Not more than 0·3 O.D.

* : Above than the permissible limit.

: The extractants not showed in the figure did not contribute to the leaching of u.v. absorbing materials in detectable amounts

: Values are the mean of four samples.

Fig. 13 Influence of storage time on the migration of u.v. absorbing materials in O.D. from blood bags of A & B brands at 25°C.

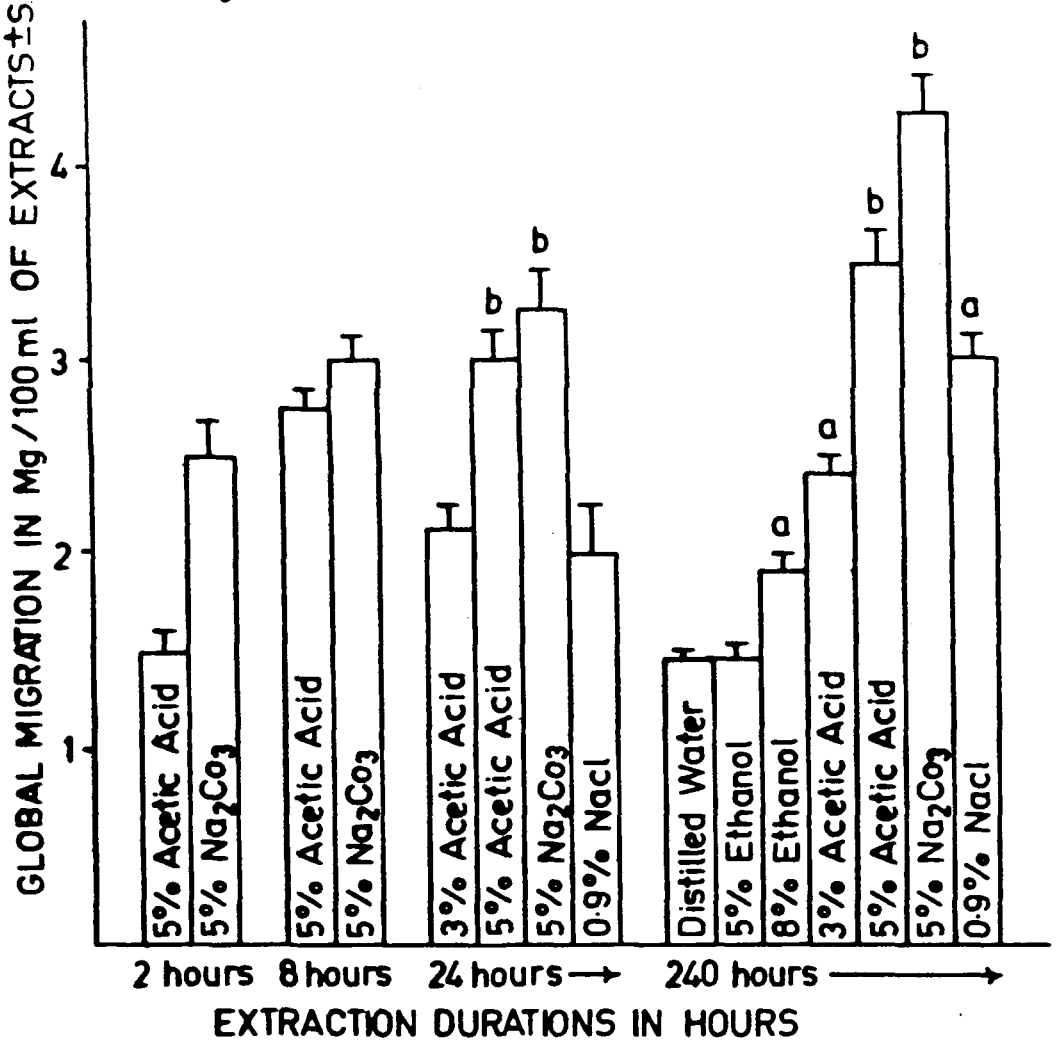


Permissible limit (PL) : Not more than 0·3 O.D.

: The extractants and extracting conditions not showed in the figure did not contribute to the leaching of u.v. absorbing materials in detectable amounts.

: Values are the mean of four samples.

Fig 14. Influence of storage time on the global migration in different extracting media from freeze bottles of A brand at 25°c.



a : Significant increase of global migration in comparison with distilled water.

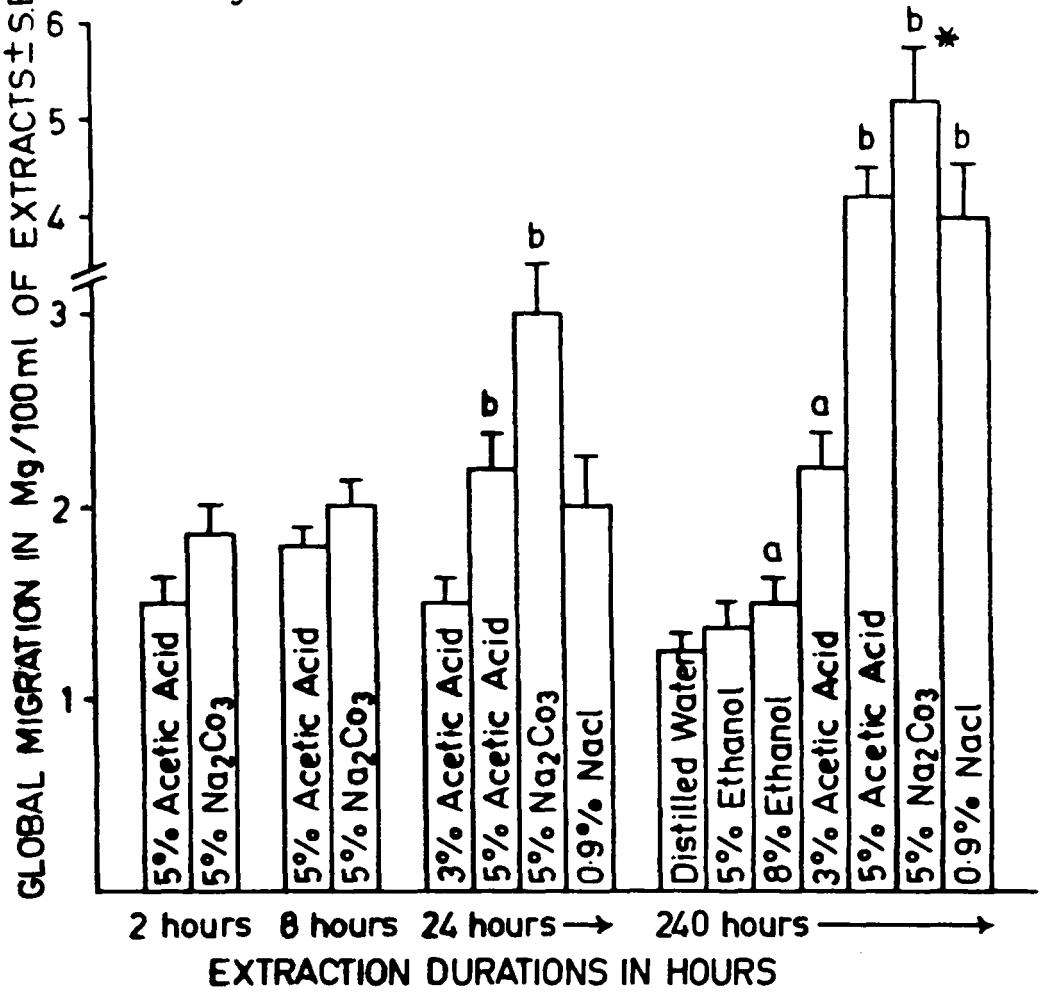
b : Significant increase of global migration in comparison with 3% acetic acid.

: The extractants and extracting conditions not showed in the figure did not contribute global migration in detectable amounts.

: Values are mean ± S. E. of four samples, $P < 0.05$ was considered to be significant, (Student's 't' test)

PL : Should not exceed 5 mg /100 ml of extracts.

Fig:15. Influence of storage time on the global migration in different extracting media from freeze bottles of B brand at 25°c.



* : Above than the permissible limit. (PL)

a : Significant increase of global migration in comparison with distilled water.

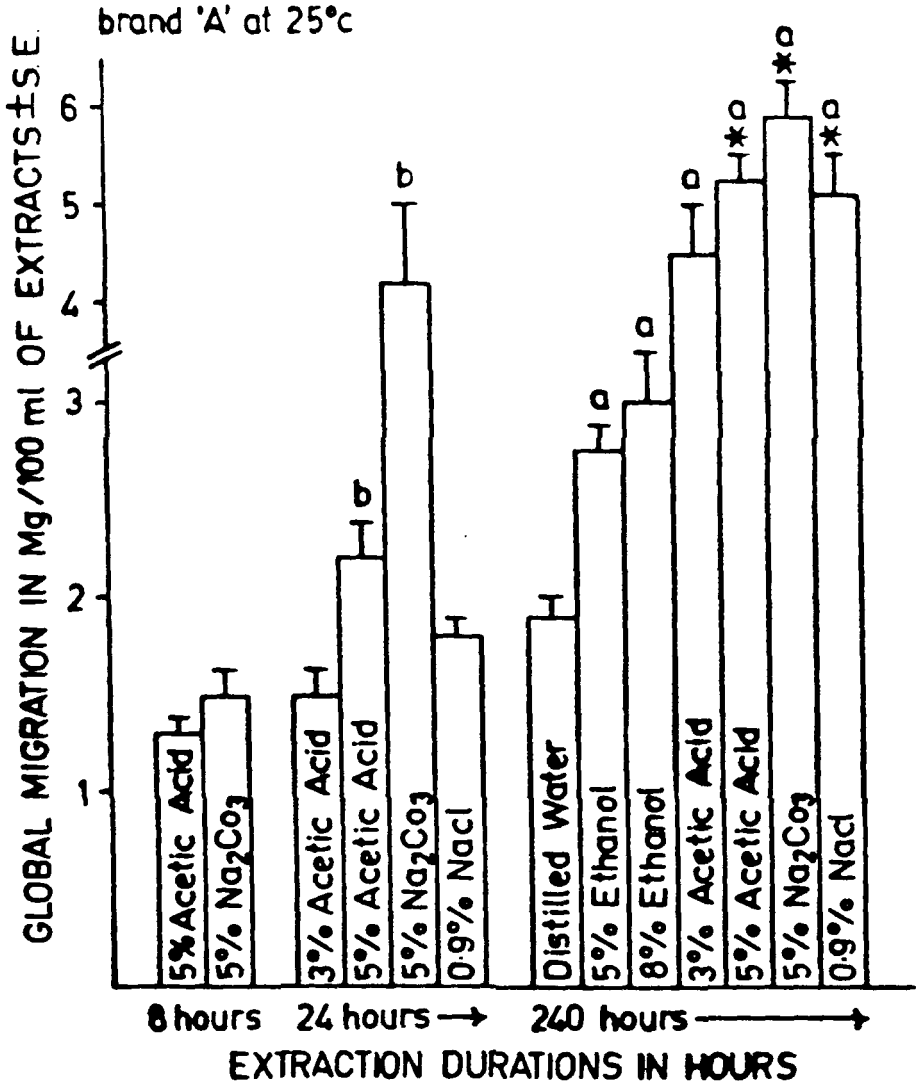
b : Significant increase of global migration in comparison with 3% acetic acid.

: The extractants and extracting conditions not showed in the figure did not contribute global migration in detectable amounts.

: Values are mean ± S. E. of four samples, $P < 0.05$ was considered to be significant, (Student's 't' test)

PL : Should not exceed 5 mg/100 ml of extracts.

Fig 16 Influence of storage time on the global migration in different extracting media from water tumblers of brand 'A' at 25°c



* : Above than the permissible limit. (PL)

a : Significant increase of global migration in comparison with distilled water.

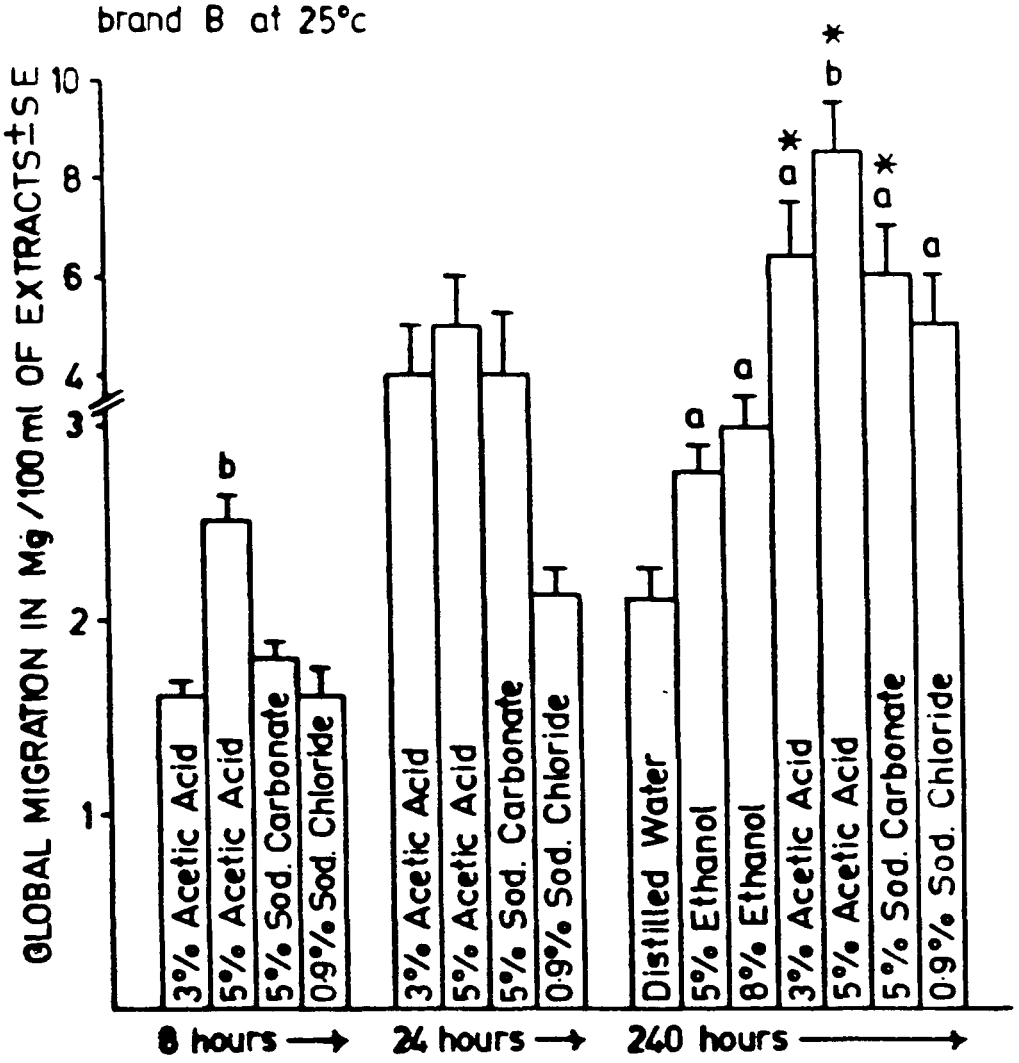
b : Significant increase of global migration in comparison with 3% acetic acid.

: The extractants and extracting conditions not showed in the figure did not contribute global migration in detectable amounts.

: Values are mean ± S.E. of four samples, $P < 0.05$ was considered to be significant, (Student's 't' test)

PL : Should not exceed 5 mg/100 ml of extracts.

Fig 17 Influence of storage time on the global migration in different extracting media from water tumblers of brand B at 25°c



* : Above than the permissible limit. (PL)

a : Significant increase of global migration in comparison with distilled water.

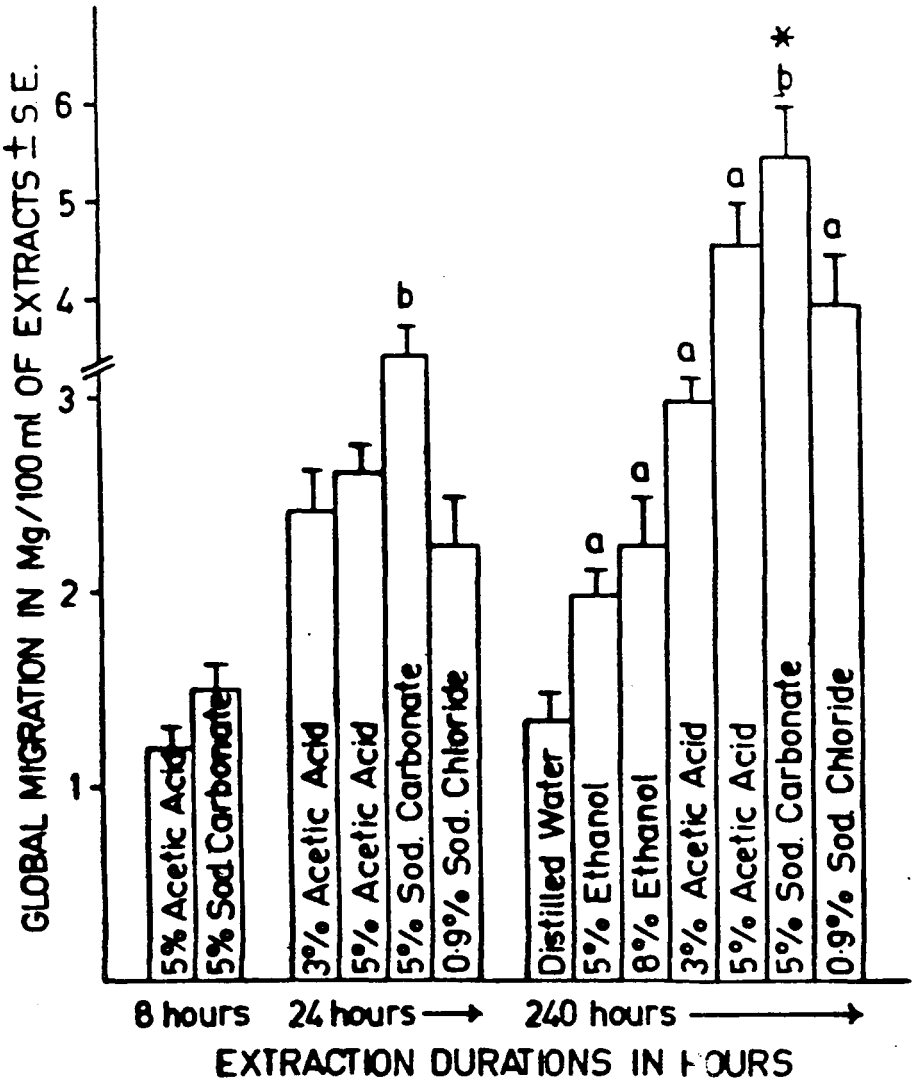
b : Significant increase of global migration in comparison with 3% acetic acid.

: The extractants and extracting conditions not showed in the figure did not contribute global migration in detectable amounts.

: Values are mean ±S.E. of four samples, $P < 0.05$ was considered to be significant, (Student's 't' test)

PL : Should not exceed 5 mg/100 ml of extracts.

Fig. 18. Influence of storage time on the global migration in different extracting media from lunch boxes of A brand at 25°C.



* : Above than the permissible limit. (PL)

a : Significant increase of global migration in comparison with distilled water.

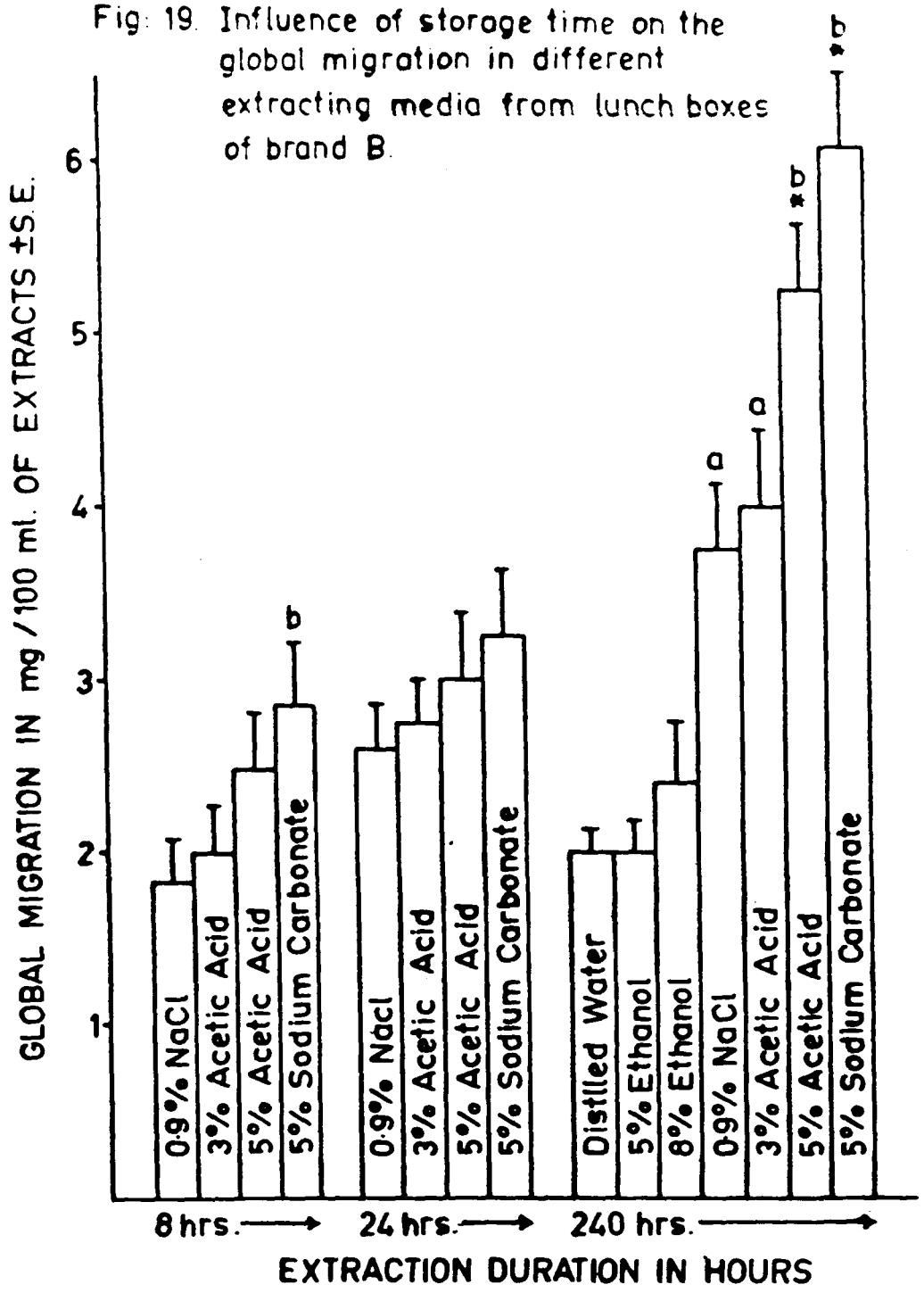
b : Significant increase of global migration in comparison with 3% acetic acid.

: The extractants and extracting conditions not showed in the figure did not contribute global migration in detectable amounts.

: Values are mean ± S.E. of four samples, $P < 0.05$ was considered to be significant, (Student's 't' test)

PL : Should not exceed 5 mg / 100 ml of extracts.

Fig: 19. Influence of storage time on the global migration in different extracting media from lunch boxes of brand B.



* : Above than the permissible limit. (PL)

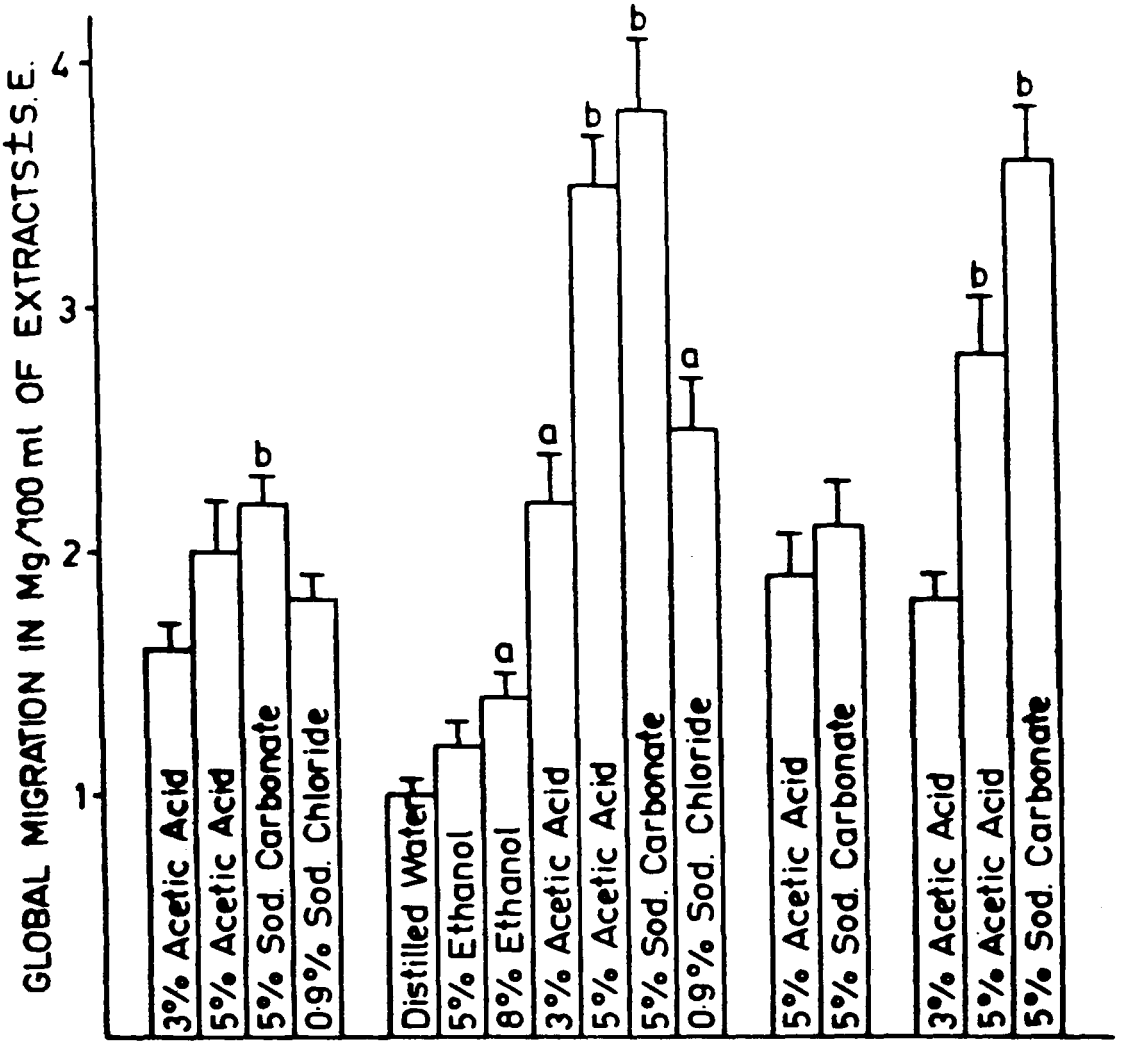
a : Significant increase of global migration in comparison with distilled water.

b : Significant increase of global migration in comparison with 3% acetic acid.

: The extractants and extracting conditions not showed in the figure did not contribute global migration in detectable amounts.

: Values are mean ±S.E. of four samples, $P \leq 0.05$ was considered to be significant, (Students 't' test.

Fig.20 Influence of storage time on the global migration in different extracting media from blood bags of A & B brands at 25°c.



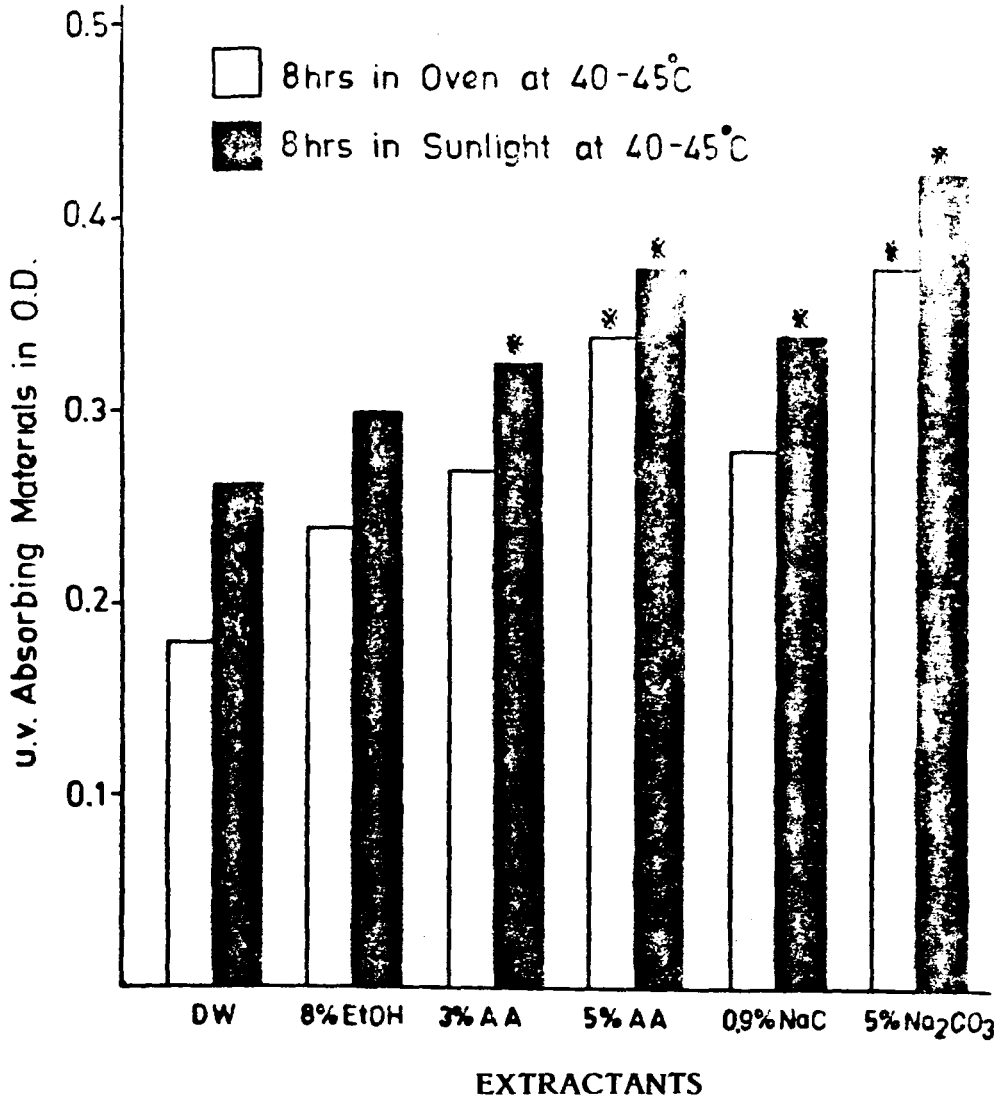
a : Significant increase of global migration in comparison with distilled water.

b : Significant increase of global migration in comparison with 3% acetic acid.

: The extractants and extracting conditions not showed in the figure did not contribute global migration in detectable amounts.

: Values are mean ±S.E. of four samples, $P < 0.05$ was considered to be significant, (Student's 't' test)

Figure - 21: Effect of sunlight on the migration of U.V. absorbing materials from freeze bottles of brand A.

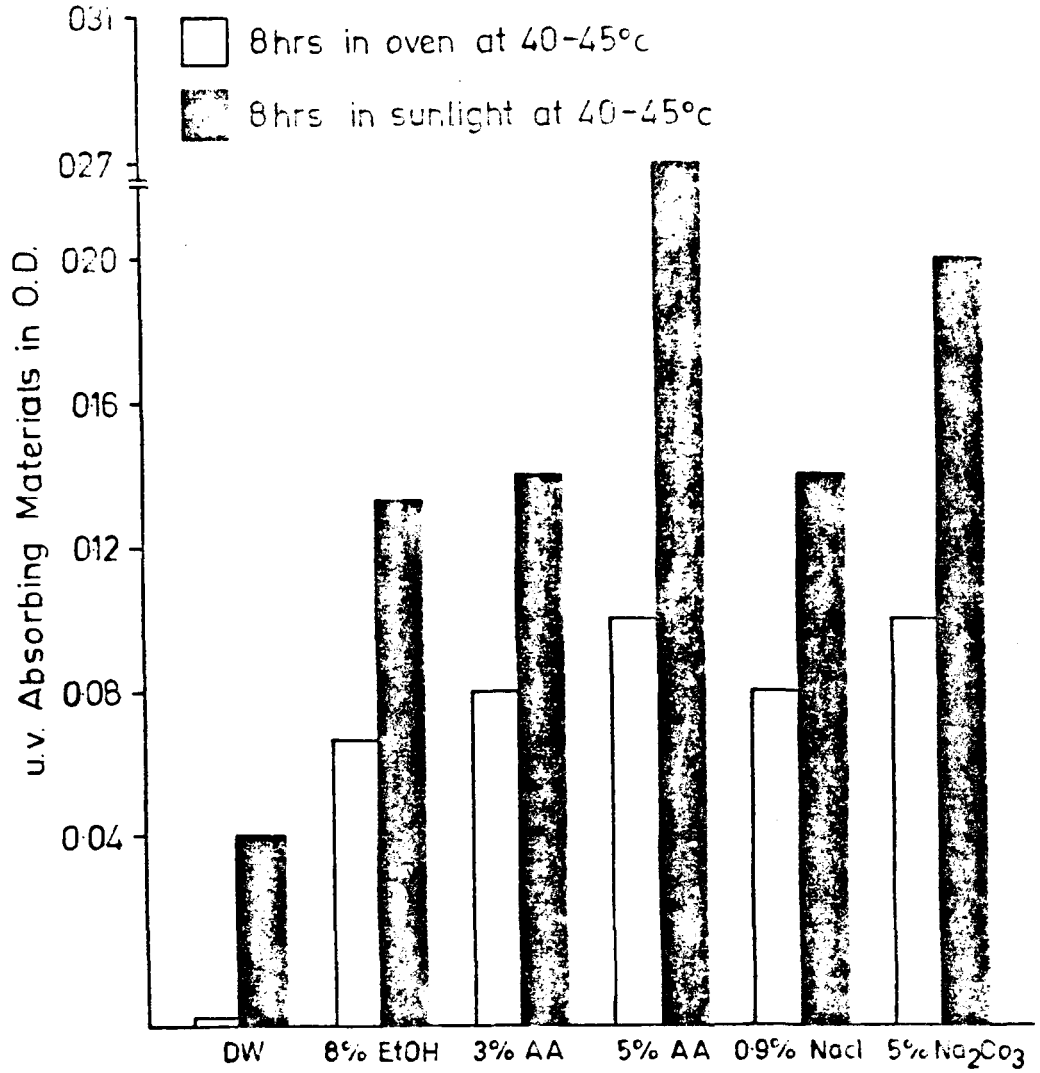


Permissible limit (PL): Not more than 0.3 OD

: Above than the permissible limit.

: Values are the mean of four samples.

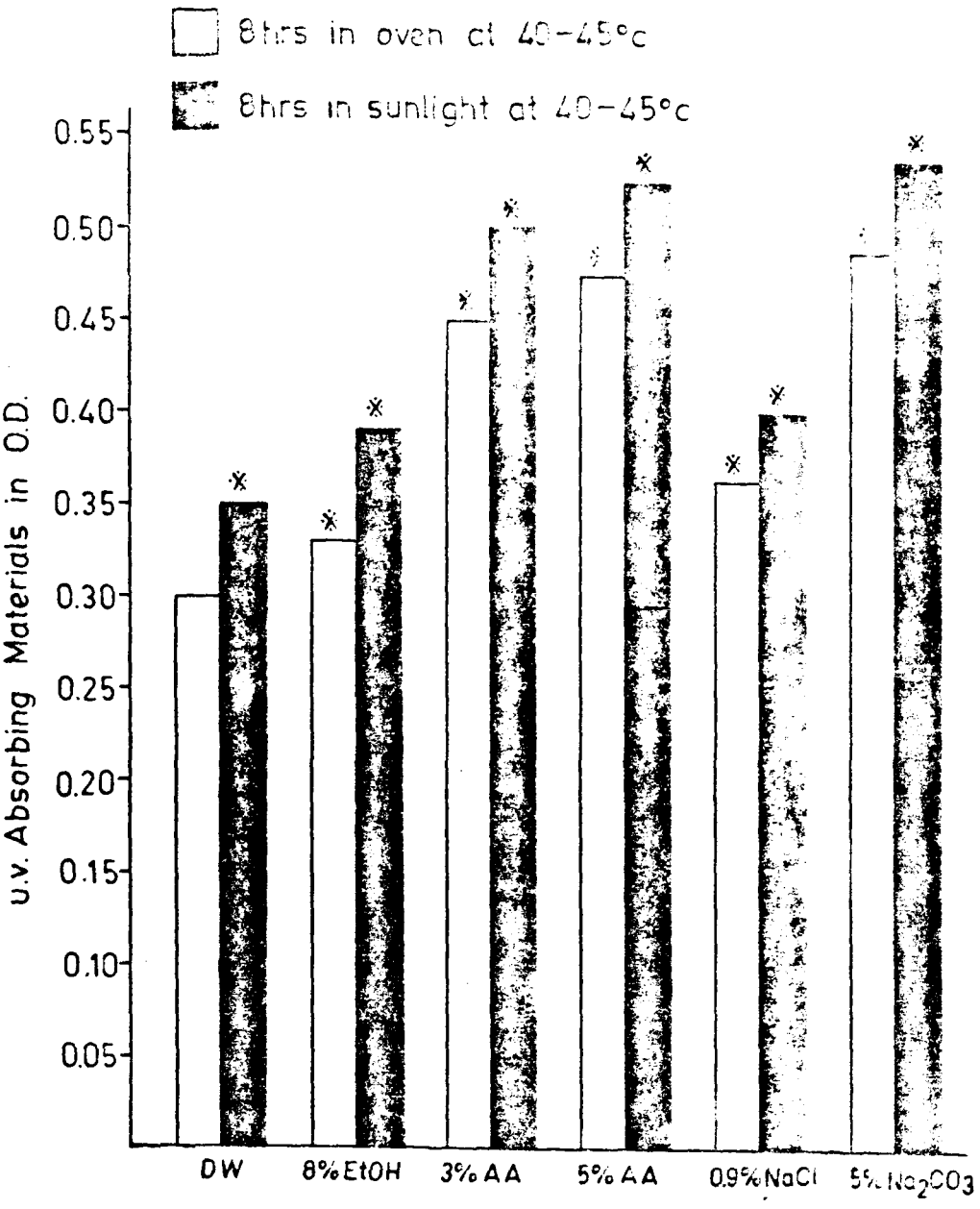
Figure - 22: Effect of sunlight on the migration of U.V. absorbing materials from freeze bottles of brand B.



Permissible limit (PL): Not more than 0.3 OD

: Values are the mean of four samples.

Figure - 23 Effect of sunlight on the migration of U.V. absorbing materials from water tumblers of brand A.

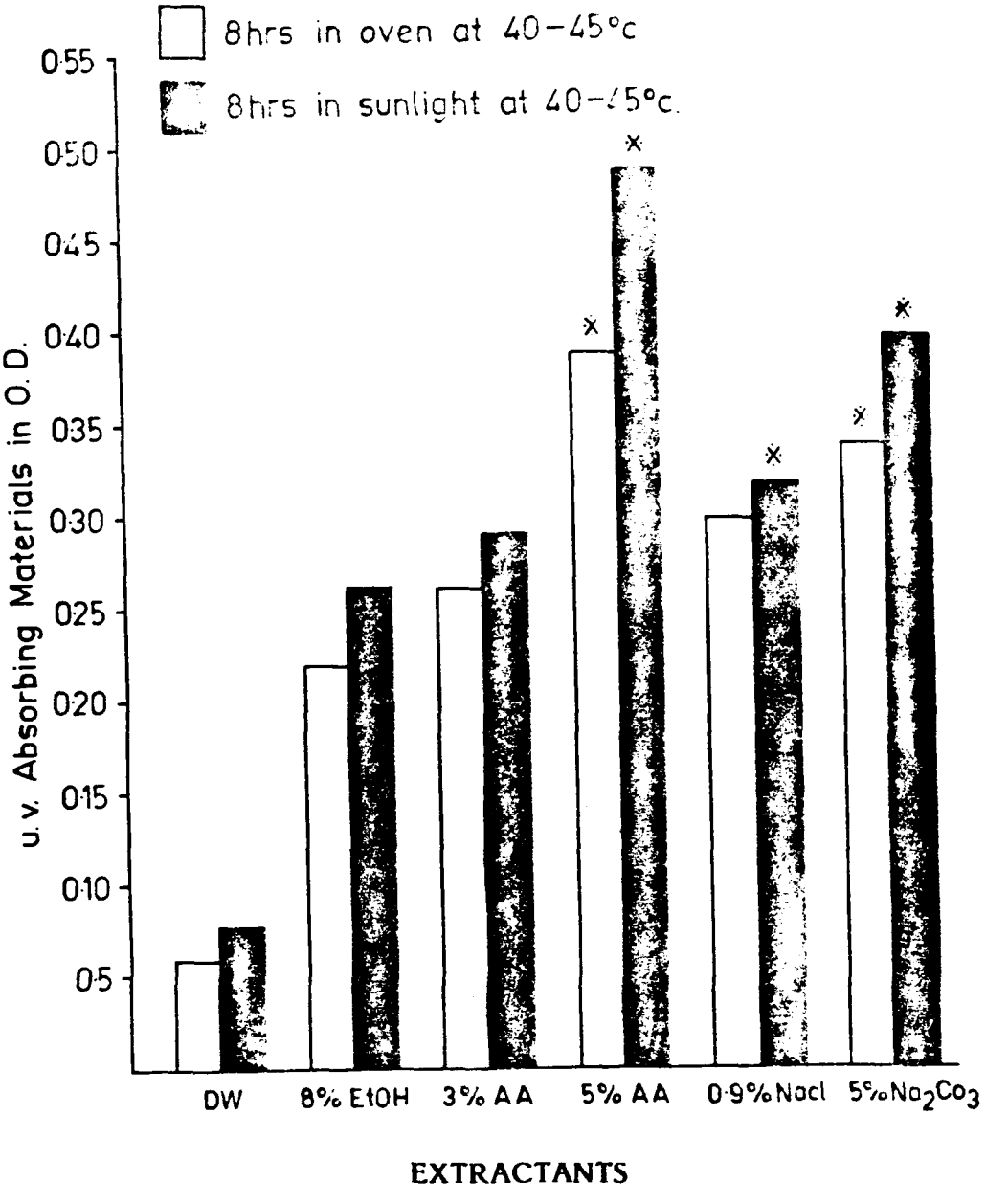


Permissible limit (PL) : Not more than 0.3 OD

* : Above than the permissible limit.

* : Values are the mean of four samples.

Figure - 24: Effect of sunlight on the migration of U.V. absorbing materials from water tumblers of brand B.

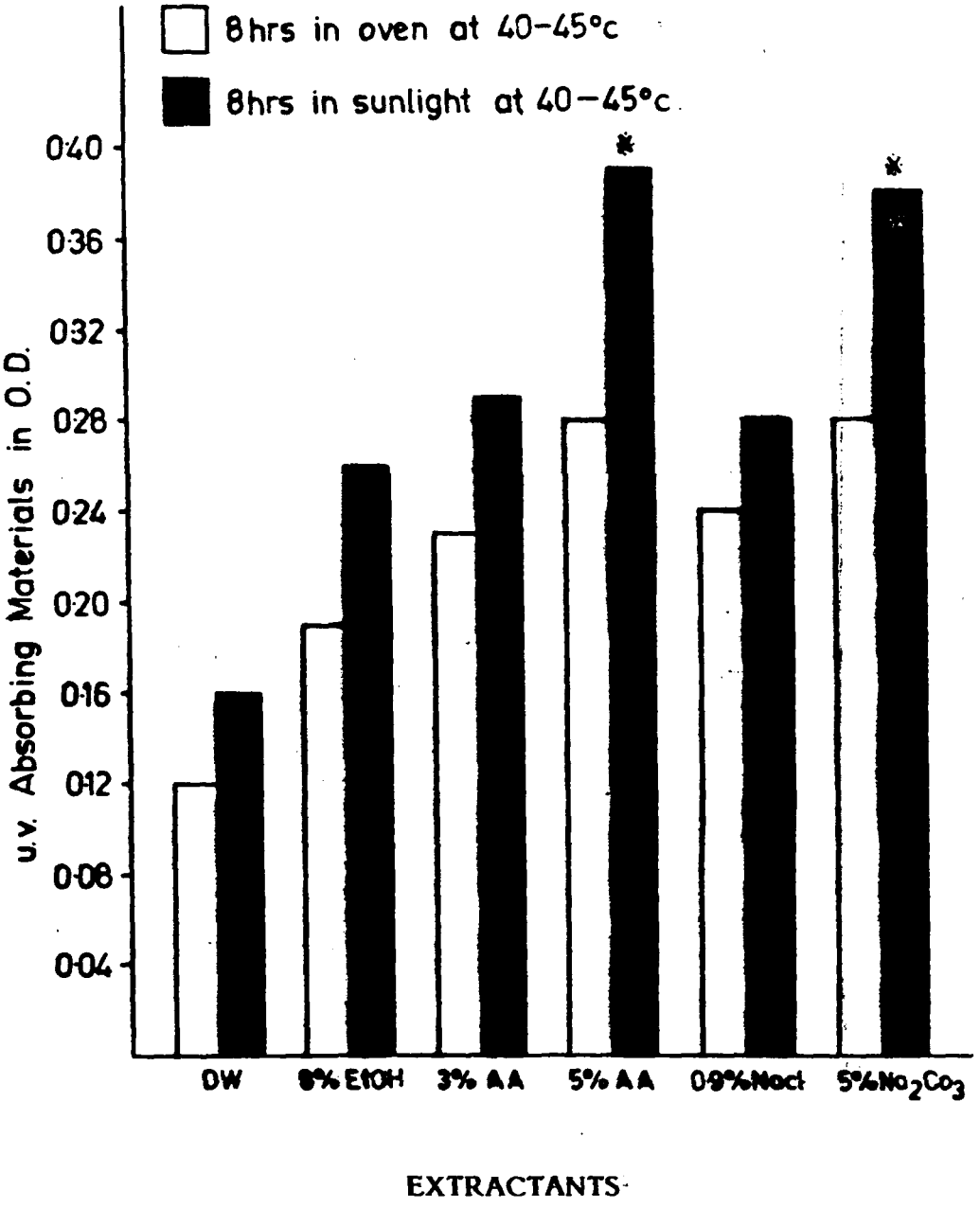


Permissible limit (PL) : Not more than 0.3 OD

* : Above than the permissible limit.

: Values are the mean of four samples.

Figure - 25: Effect of sunlight on the migration of U.V. absorbing materials from lunch boxes of brand A.

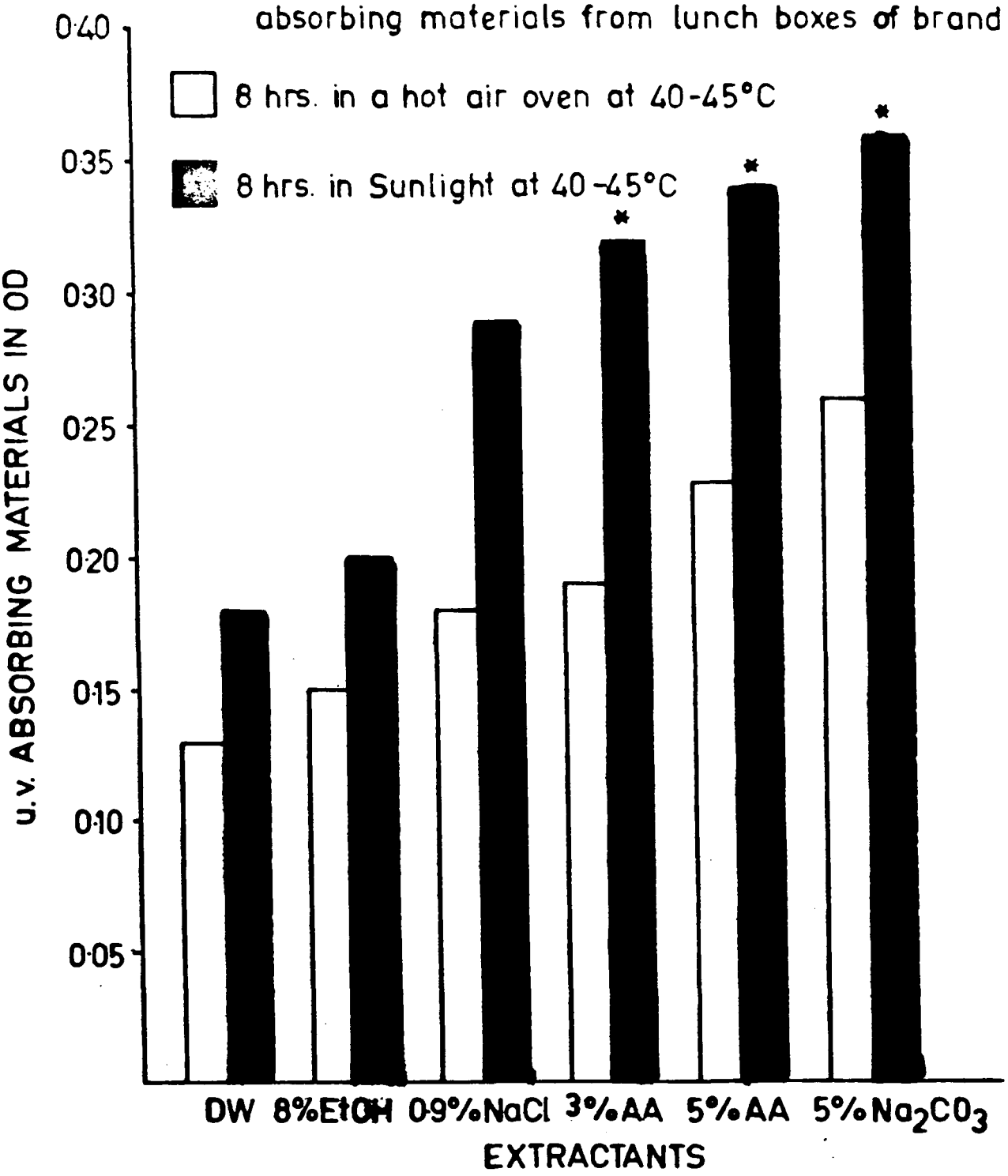


Permissible limit (PL) : Not more than 0.3 OD

* : Above than the permissible limit.

: Values are the mean of four samples.

Fig: 26. Effect of Sunlight on the migration of u.v. absorbing materials from lunch boxes of brand B.

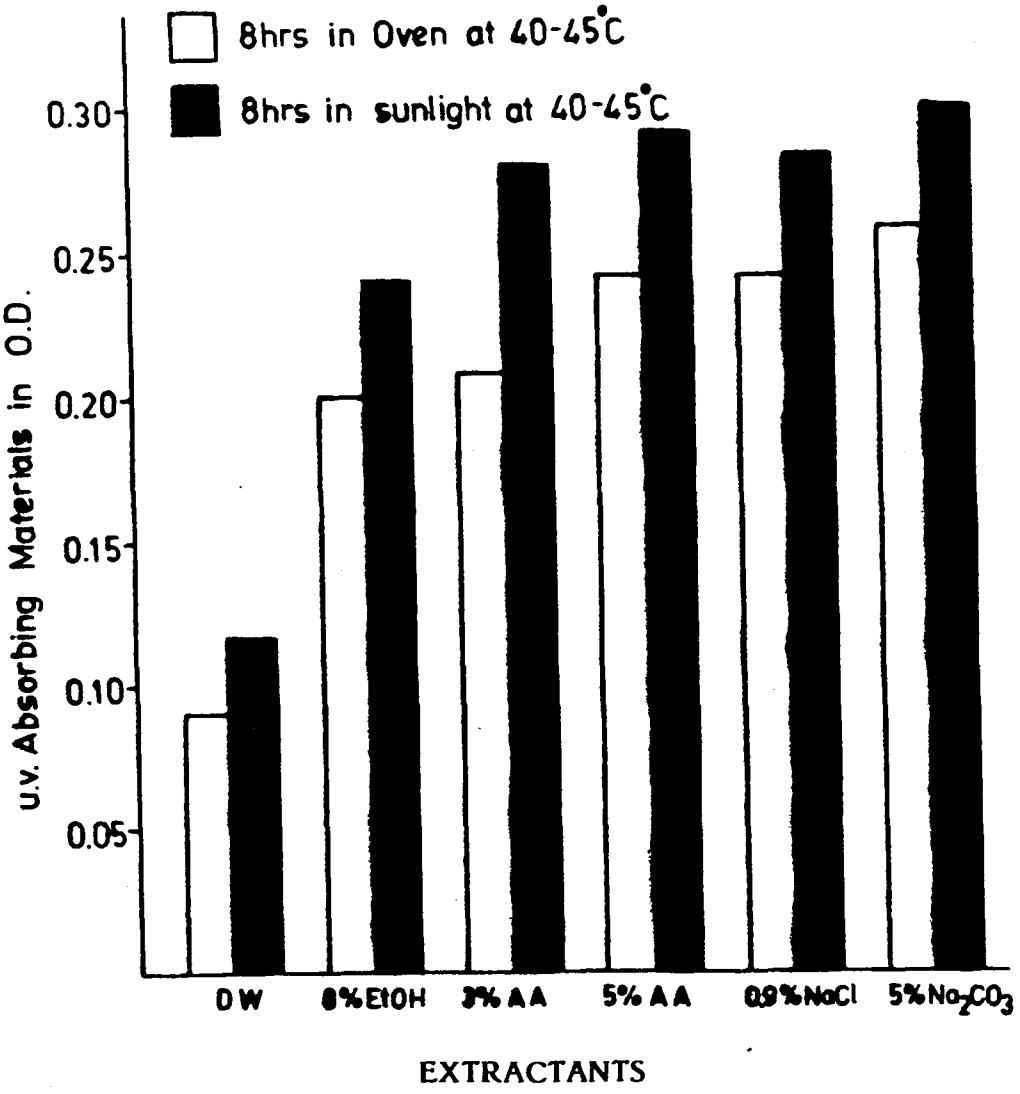


Permissible limit (PL) : Not more than 0·3 OD

* : Above than the permissible limit.

: Values are the mean of four samples.

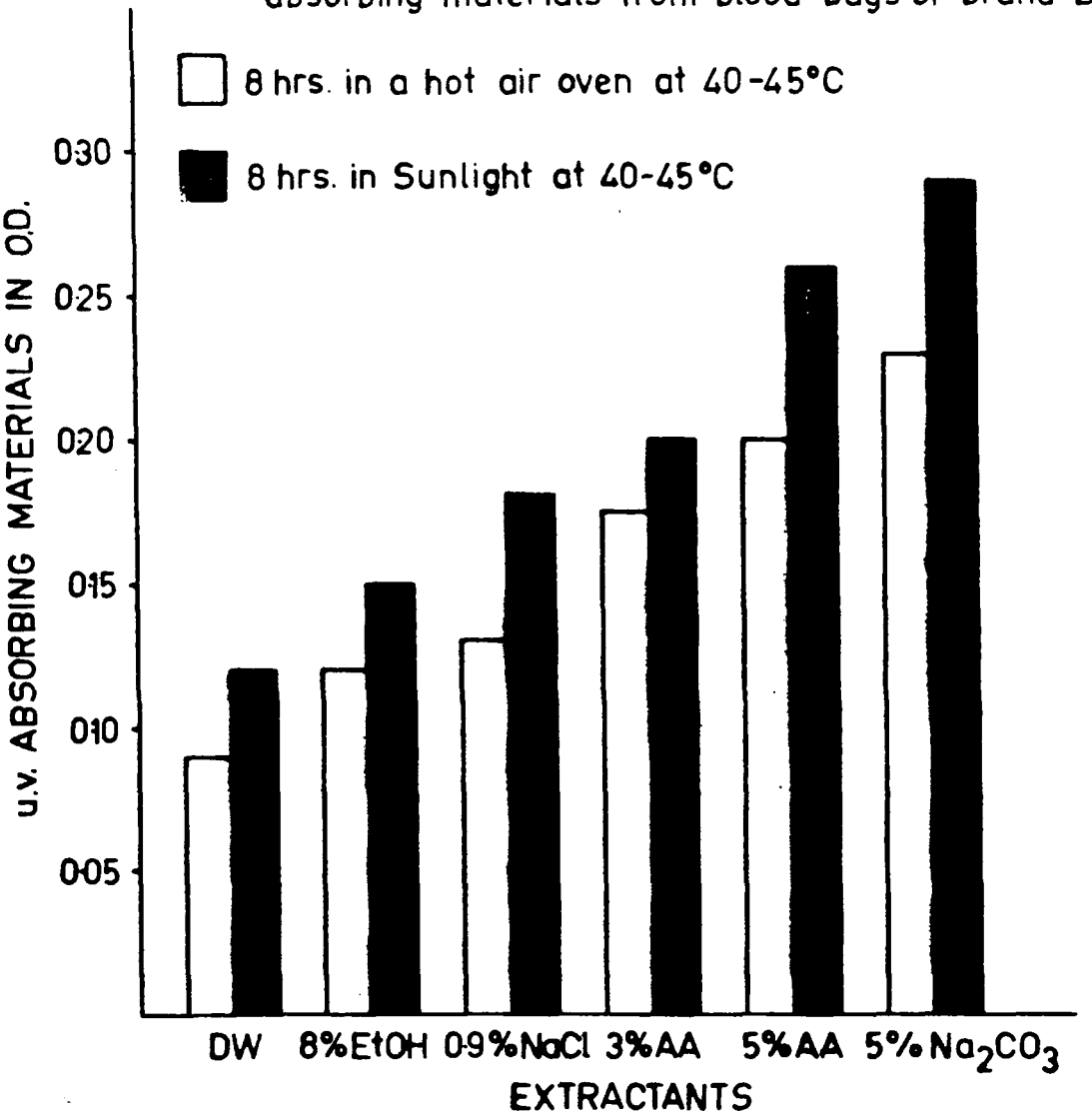
Figure - 27: Effect of sunlight on the migration of U.V. absorbing materials from blood bags of brand A.



Permissible limit (PL) : Not more than 0.3 OD

* : Values are the mean of four samples.

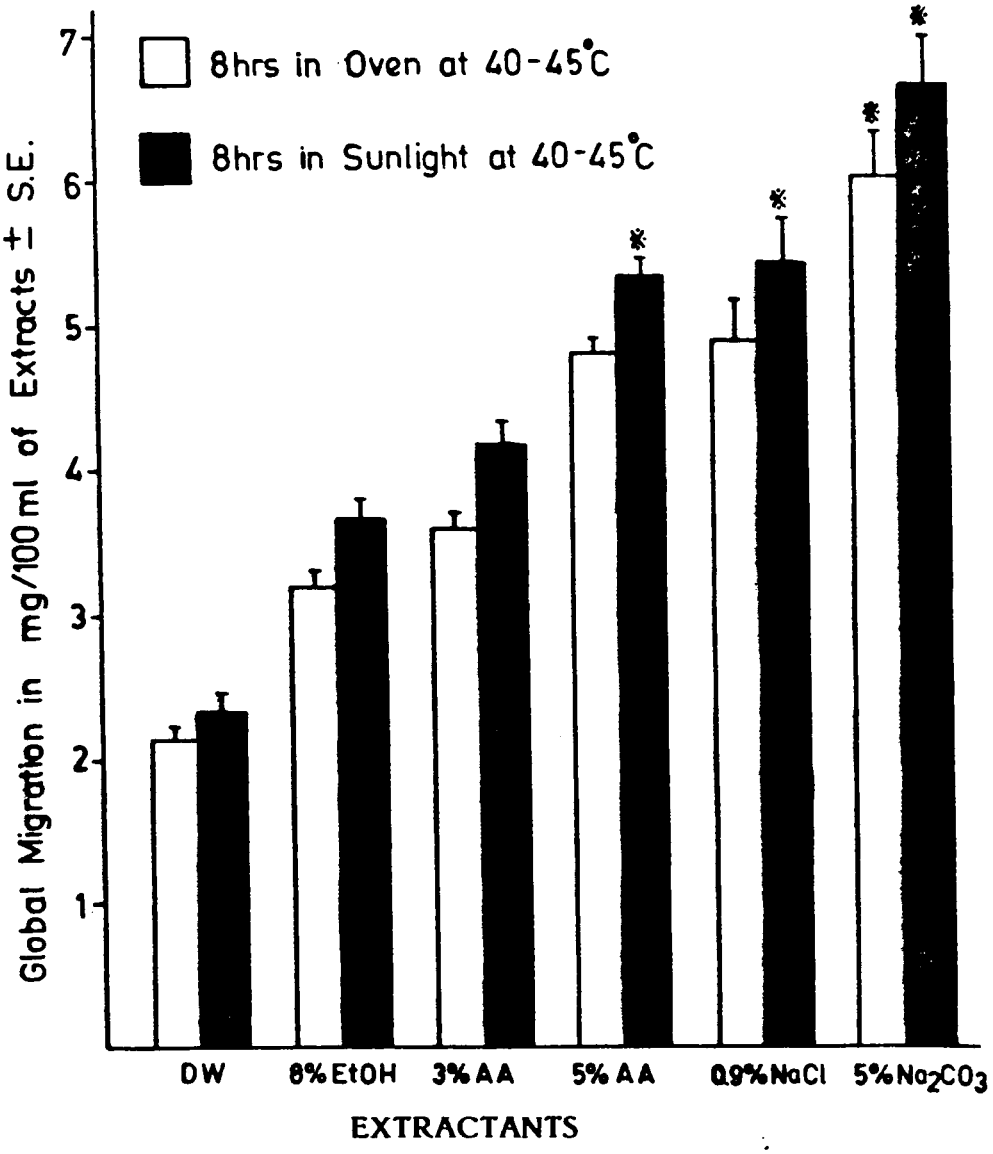
Fig: 28. Effect of Sunlight on the migration of u.v. absorbing materials from blood bags of brand B.



Permissible limit (PL) : Not more than 0·3 OD

: Values are the average of four samples.

Figure - 29: Effect of sunlight on the global migration from freeze bottles of brand A.

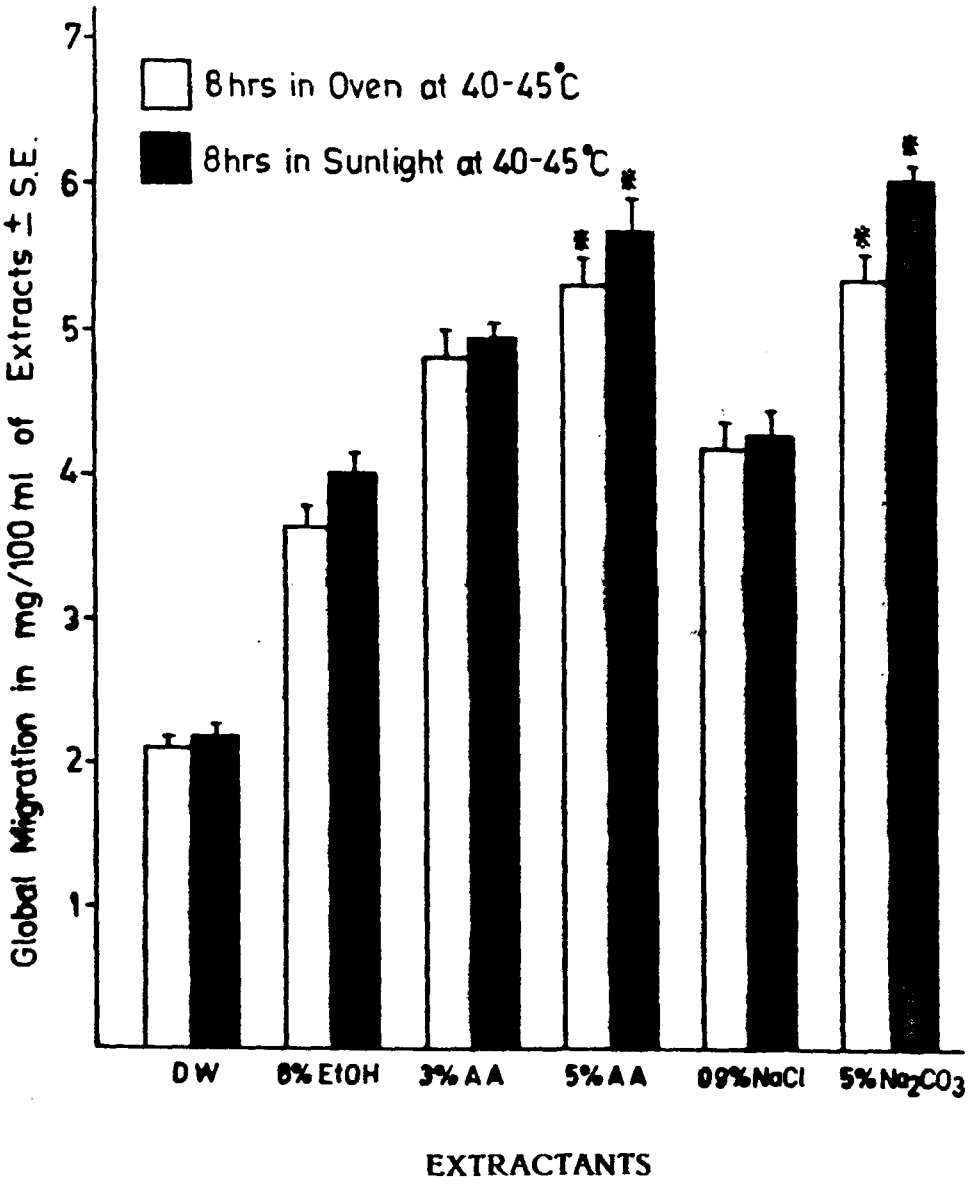


Permissible limit (PL) : Residue in 100 ml of extracts should not exceed 5 mg.

* : Above than the permissible limit.

: Values are the mean ±S.E. of four samples p 0.05 was considered to be significant (student's 't' test)

Figure - 30: Effect of sunlight on the global migration from freeze bottles of brand B.



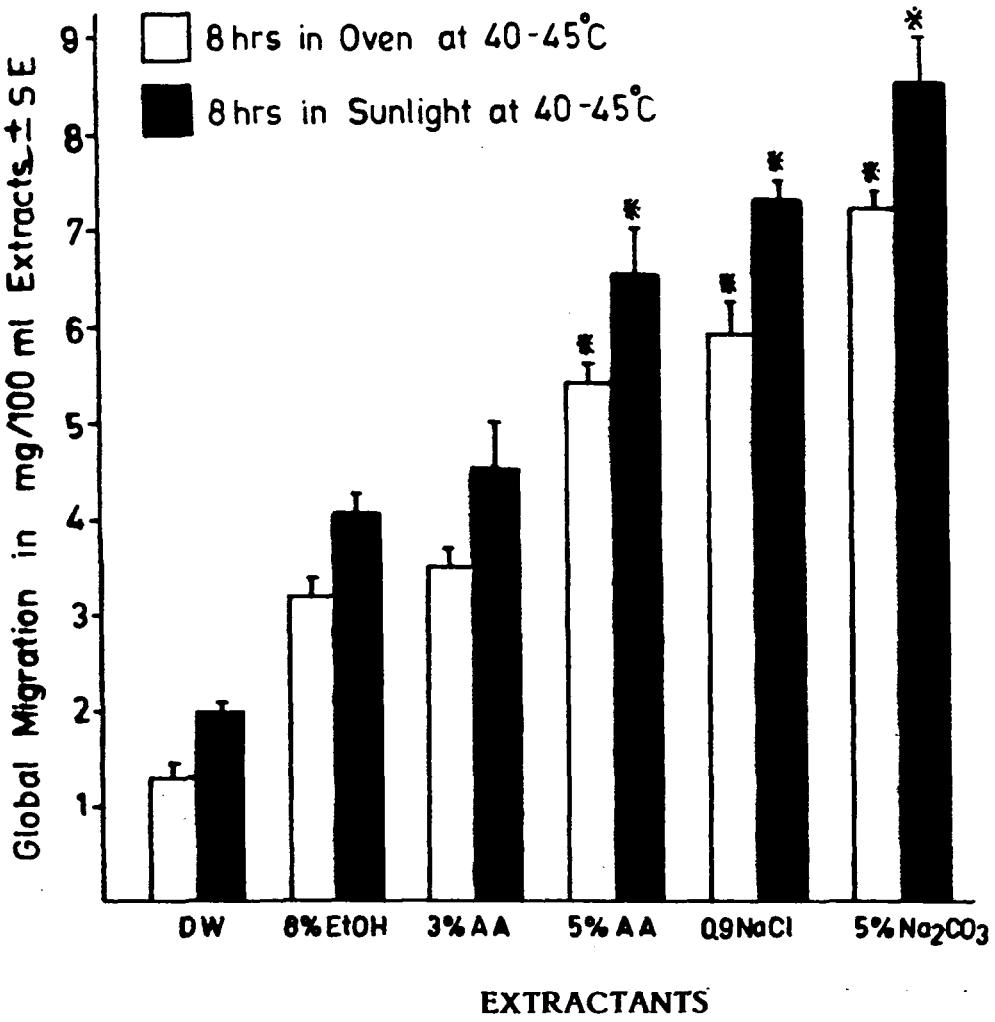
Permissible limit (PL) : Residue in 100 ml of extracts should not exceed 5 mg.

* : Above than the permissible limit.

: Values are the mean ±S.E. of four samples

p 0.05 was considered to be significant (student's 't' test)

Figure - 31: Effect of sunlight on the global migration from water tumblers of brand A.

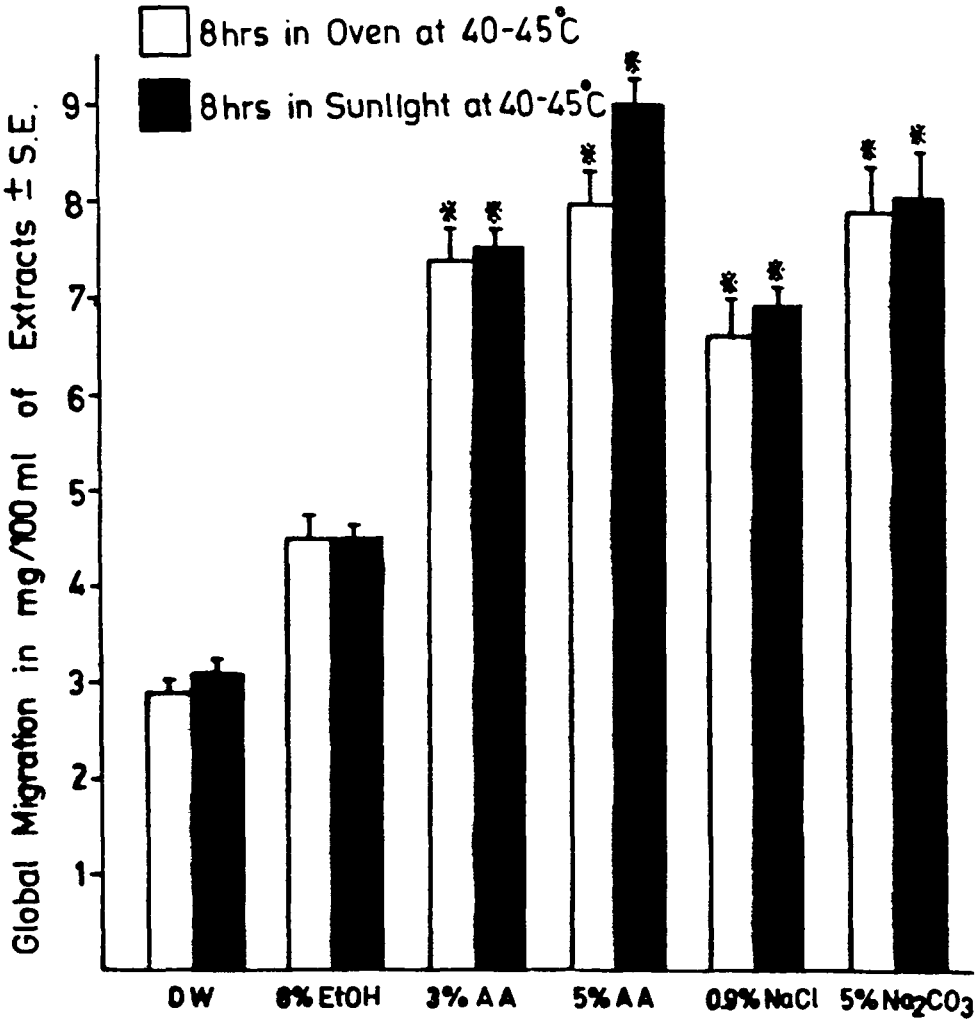


Permissible limit (PL) : Residue in 100 ml of extracts should not exceed 5 mg.

* : Above than the permissible limit.

: Values are the mean ±S.E. of four samples p 0.05 was considered to be significant (student's 't' test).

Figure - 32: Effect of sunlight on the global migration from water tumblers of brand B.



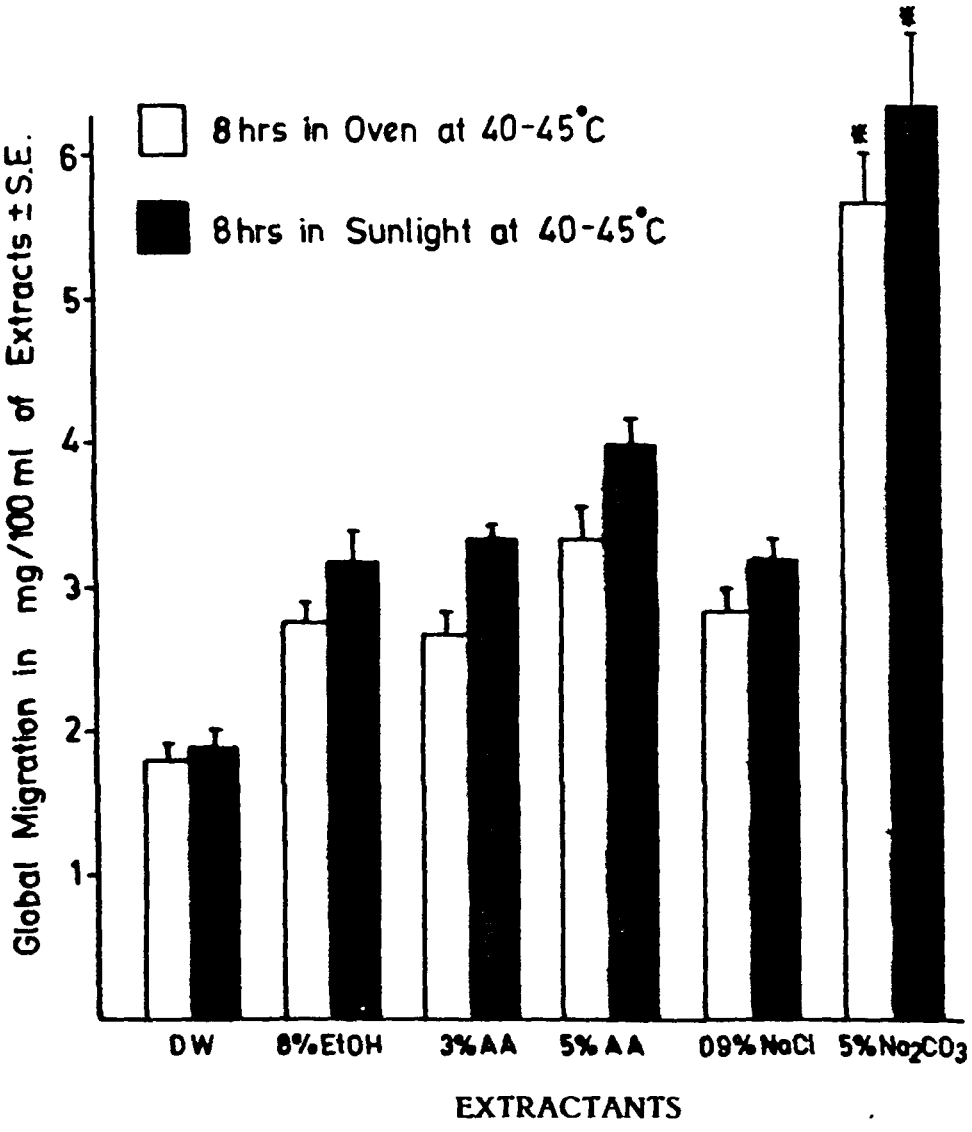
Permissible limit (PL) : Residue in 100 ml of extracts should not exceed 5 mg.

* : Above than the permissible limit.

: Values are the mean ±S.E. of four samples

p 0.05 was considered to be significant (student's 't' test).

Figure - 33: Effect of sunlight on the global migration from lunch boxes of brand A.

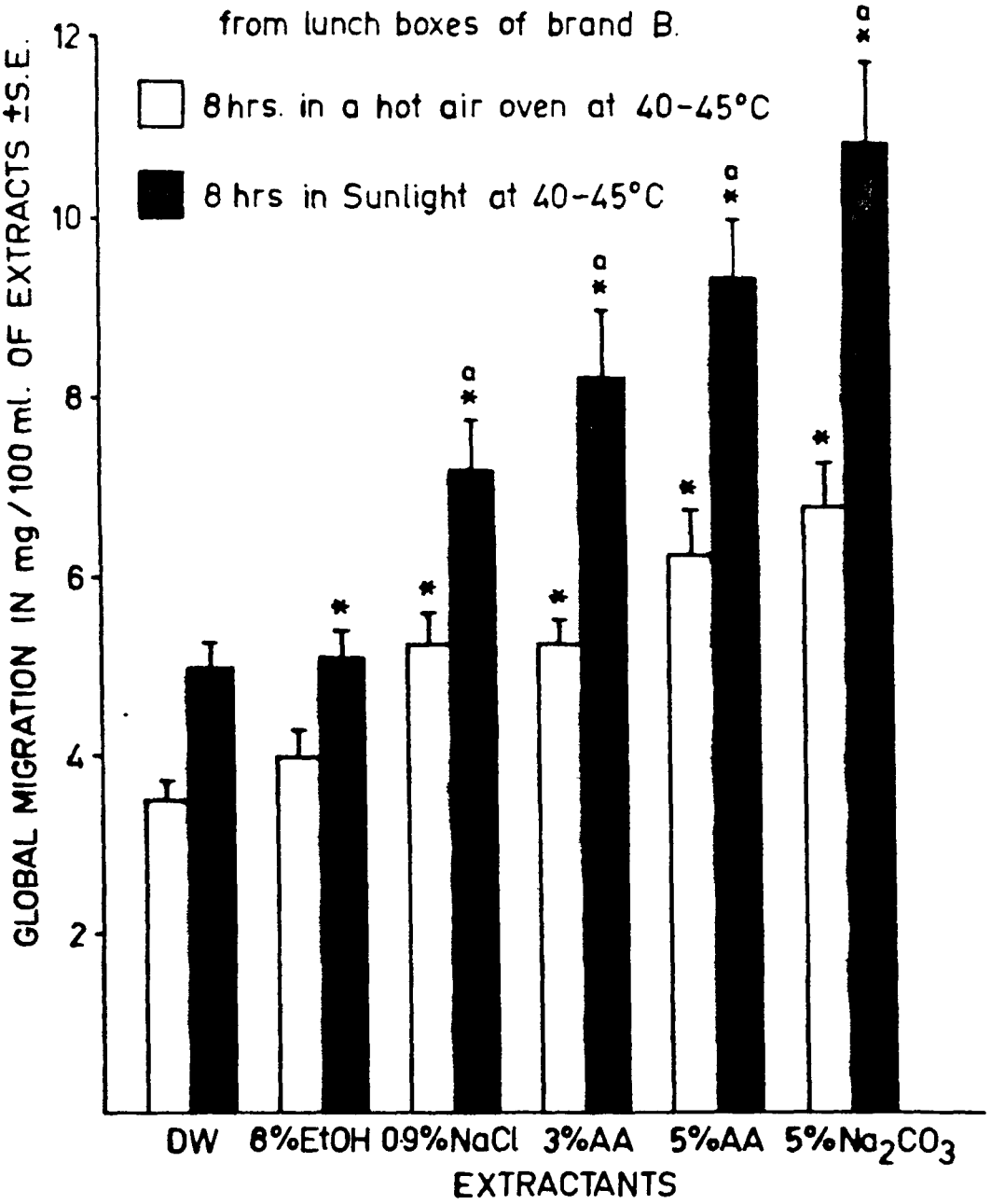


Permissible limit(PL) : Residue in 100 ml of extracts should not exceed 5 mg.

* : Above than the permissible limit.

: Values are the mean ±S.E. of four samples p 0.05 was considered to be significant (student's 't' test).

Fig: 34 Effect of Sunlight on the global migration from lunch boxes of brand B.



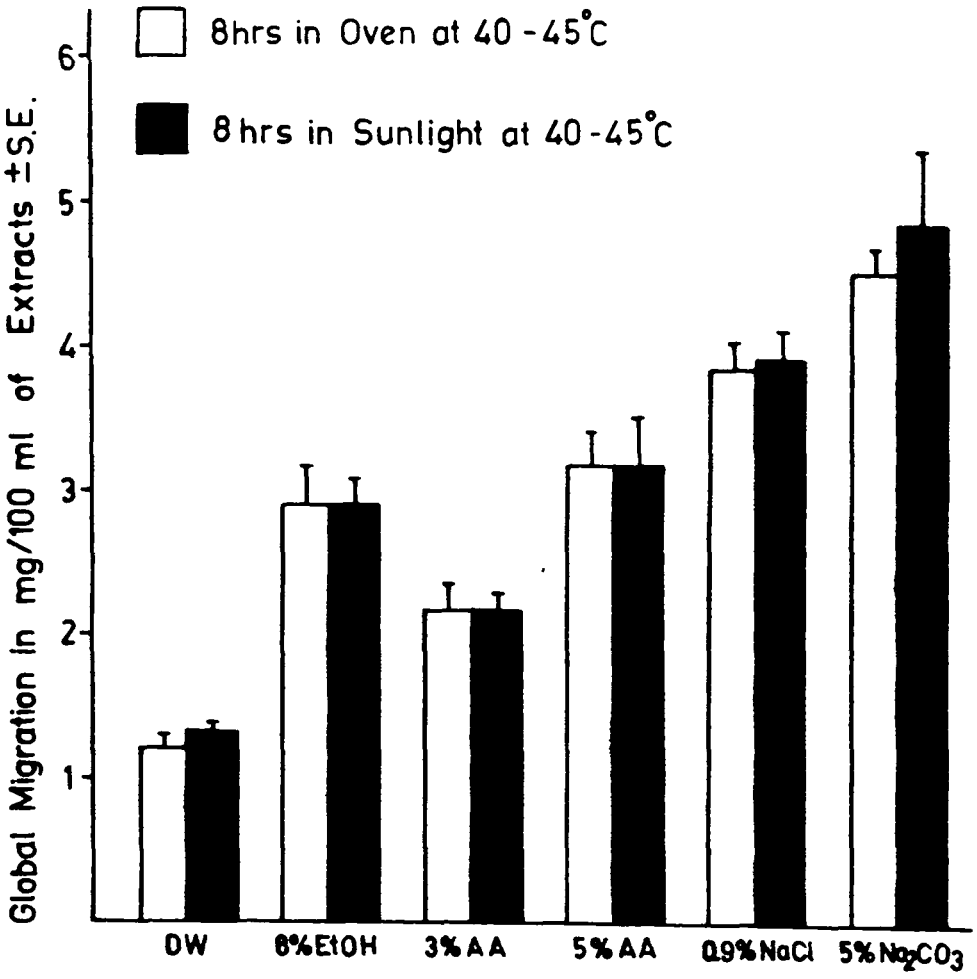
Permissible limit (PL) : Residue in 100 ml of extracts should not exceed 5 mg.

* : Above than the permissible limit.

: Values are the mean ±S.E. of four samples ±S.E., $p < 0.05$. was considered to be significant (student's 't' test)

a : Significant increase of global migration under sunlight in comparison with the migration obtained at the same temperature in the hot air oven.

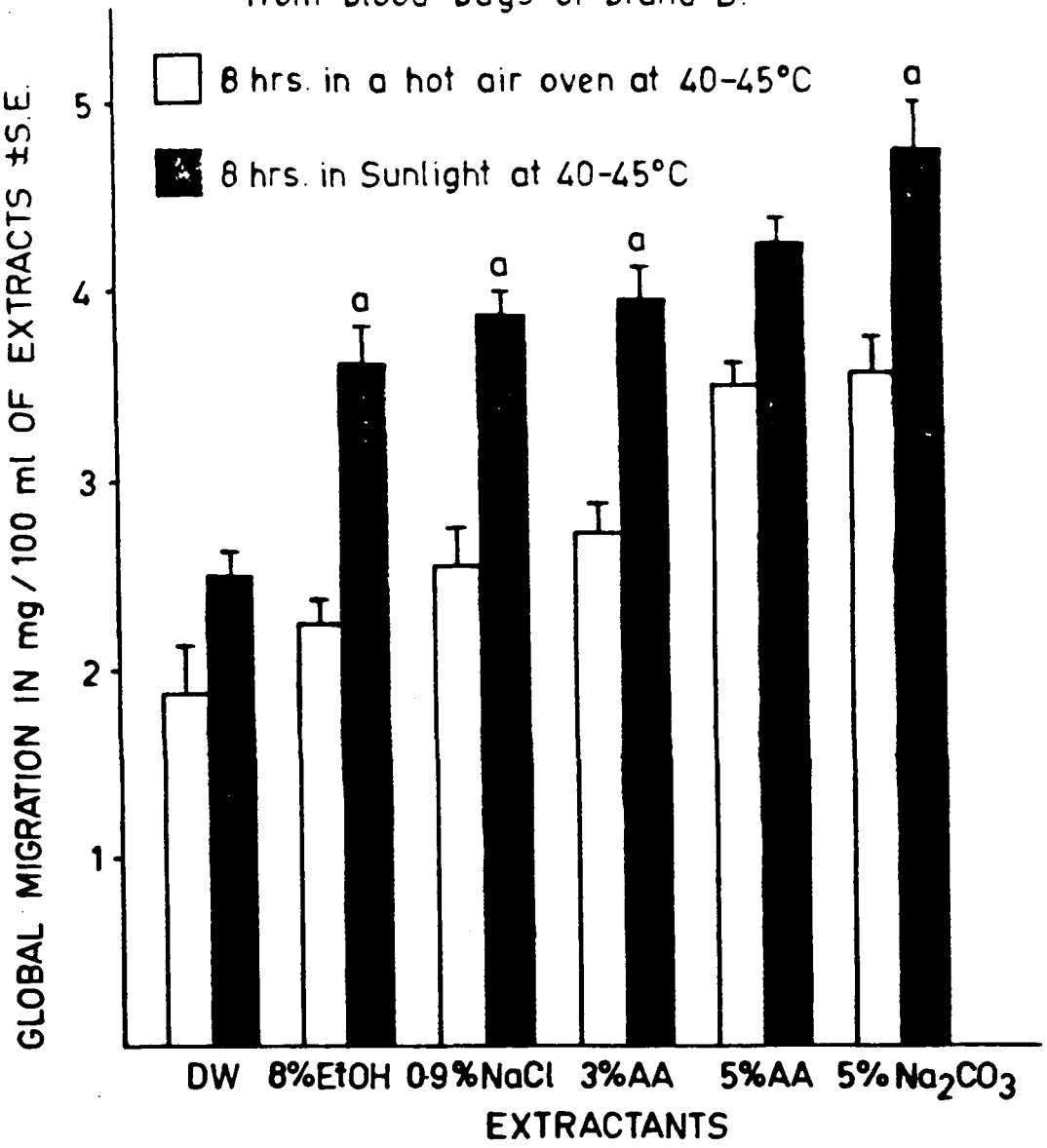
Figure - 35 : Effect of sunlight on the global migration from blood bags of brand A.



Permissible limit(PL) : Residue in 100 ml of extracts should not exceed 5 mg.

: Values are the mean ±S.E. of four samples p 0.05 was considered to be significant (student's 't' test).

Fig: 36. Effect of Sunlight on the global migration from blood bags of brand B.



Permissible limit (PL) : Residue in 100 ml of extracts should not exceed 5 mg.

a : Significant increase of global migration under Sunlight in comparison with the migration obtained at the same temperature in the hot air oven.

: Values are the mean of four samples ± S.E., $p < 0.05$ was considered to be Significant (student's 't' test).

Table - 15: Influence of Sunlight on the migration of heavy metals (APL, in ppm) from freeze bottles of Brand-A.

Extractants	8 hours in hot air oven at 40-45°C	8 hours in sunlight at 40-45°C
Distilled water	NAPL	Cd(0.20±0.02)
8% Ethanol	NAPL	NAPL
3% Acetic Acid	NAPL	Cd(0.45±0.05)
5% Acetic Acid	Cd(0.20±0.01)	Cd(0.65±0.03)[a]
5% Sodium Carbonate	Cd(0.34±0.03)	Cd(0.86±0.02)[a]
0.9% Sodium Chloride	NAPL	NAPL

Permissible limit (PL): Concentrations of Cr, Cu, Mn, Zn & Pb in the plastic extracts should not exceed 1 ppm and that of Cd should not exceed 0.1 ppm.

APL : Above than the permissible limit.

NAPL : None above than the permissible limit.

a : Significant increase of migration of metals under sunlight in comparison with the migrations obtained in the hot air oven at the same temperature.

: Values are mean ±SE of four samples, $p < 0.05$ was considered to be significant (Student's 't' test).

Table - 16: Influence of Sunlight on the migration of heavy metals (APL, in ppm) from freeze bottles of Brand 'B'

Extractants	8 hours in hot air oven at 40-45°C	8 hours in sunlight at 40-45°C
Distilled water	NAPL	Pb(1.80 ±0.10) Mn(1.90 ±0.04)
8% Ethanol	NAPL	NAPL
3% Acetic Acid	Pb(1.20 ±0.03)	Pb(2.30 ±0.20]^{a} Mn(2.10 ±0.10) Zn(1.40 ±0.05)^{a}
5% Acetic Acid	Pb(2.46 ±0.06) Zn(1.40 ±0.01)	Pb[4.90 ±0.00)^{a} Mn(2.40 ±0.08) Zn(2.00 ±0.20)^{a}
5% Sodium Carbonate	Cd(0.30 ±0.00)	Pb(2.40 ±0.04) Cd(0.80 ±0.12)^{a}
0.9% Sodium Chloride	NAPL	Pb(2.00 ±0.16) Zn(1.6±0.06)

Permissible limit (PL) : Concentrations of Cr, Cu, Mn, Zn & Pb in the plastic extracts should not exceed 1 ppm and that of Cd should not exceed 0.1 ppm.

APL : Above than the permissible limit.

NAPL : None above than the permissible limit.

a : Significant increase of migration of metals under sunlight in comparison with the migrations obtained in the hot air oven at the same temperature.

: Values are mean ±SE of four samples, $p < 0.05$ was considered to be significant(Student's 't' test).

Table - 17: Influence of Sunlight on the migration of heavy metals (APL, in ppm) from water tumblers of Brand 'A'

Extractants	8 hours in hot air oven at 40-45°C	8 hours in sunlight at 40-45°C
Distilled Water	Cd(0.25±0.00)	Cd(1.00±0.10)[a] Mn(2.00±0.06)
8% Ethanol	Cd(0.30±0.03)	Cu(2.30±0.13) Cd(0.80±0.06)[a]
3% Acetic Acid	Cd(0.30±0.00)	Cd(1.00±0.06)[a] Mn(3.00±0.03) Cr(3.10±0.12)
5% Acetic Acid	Cd(1.00±0.05)	Cd(1.80±0.12)[a] Mn(3.91±0.08) Cr(3.50±0.00)
5% Sodium Carbonate	Cd(0.50±0.12)	Cd(1.60±0.03)[a] Cr(2.3±0.20)
0.9% Sodium Chloride	Cd(0.38±0.06)	Cd(1.2±0.04)[a] Cr(2.6±0.09)

Permissible limit (PL) : Concentrations of Cr, Cu, Mn, Zn & Pb in the plastic extracts should not exceed 1 ppm and that of Cd should not exceed 0.1 ppm.

APL : Above than the permissible limit.

NAPL : None above than the permissible limit.

a : Significant increase of migration of metals under sunlight in comparison with the migrations obtained in the hot air oven at the same temperature.

: Values are mean ±SE of four samples, $p < 0.05$ was considered to be significant(Student's 't' test).

Table - 18: influence of Sunlight on the migration of heavy metals (APL, in ppm) from water tumblers of Brand 'B'

Extractants	8 hours in hot air oven at 40-45°C	8 hours in sunlight at 40-45°C
Distilled water	NAPL	NAPL
8% Ethanol	NAPL	NAPL
3% Acetic Acid	Cd(0.18±0.01)	Cd(0.22±0.03)
5% Acetic Acid	Cd(0.21±0.01)	Cd(0.34±0.04)[a]
5% Sodium Carbonate	Cd(0.23±0.04)	Cd(0.40±0.02)[a]
0.9% Sodium Chloride	NAPL	Cd(0.20±0.01)

Permissible limit (PL) : Concentrations of Cr, Cu, Mn, Zn & Pb in the plastic extracts should not exceed 1 ppm and that of Cd should not exceed 0.1 ppm.

APL : Above than the permissible limit.

NAPL : None above than the permissible limit.

a : Significant increase of migration of metals under sunlight in comparison with the migrations obtained in the hot air oven at the same temperature.

: Values are mean ±SE of four samples, $p < 0.05$ was considered to be significant(Student's 't' test).

Table - 19: Influence of Sunlight on the migration of heavy metals (APL, in ppm) from lunch boxes of Brand 'A'.

Extractants	8 hours in hot air oven at 40-45°C	8 hours in sunlight at 40-45°C
Distilled water	Cd(0.20 ±0.04) Pb(1.20 ±0.04)	Cd(0.70 ±0.05)[a] Mn(1.80 ±0.04) Pb(1.50 ±0.04)[a]
8% Ethanol	NAPL	Cu(1.80 ±0.08)
3% Acetic Acid	Cd(0.60 ±0.04) Pb(1.40 ±0.06)	Cd(1.20 ±0.04)[a] Mn(2.50 ±0.04) Pb(1.80 ±0.06)[a]
5% Acetic Acid	Cd(0.70 ±0.04) Pb(1.40 ±0.06)	Cd(1.60 ±0.04)[a] Mn(2.80 ±0.06) Pb(2.10 ±0.08)[a]
5% Sodium Carbonate	Cd(0.40 ±0.04)	Cd(1.20 ±0.10)[a] Pb(2.06 ±0.08)
0.9% Sodium Chloride	Cd(0.40 ±0.05)	Cd(1.09 ±0.04)[a] Pb(1.64 ±0.00)

Permissible limit (PL) : Concentrations of Cr, Cu, Mn, Zn & Pb in the plastic extracts should not exceed 1 ppm and that of Cd should not exceed 0.1 ppm.

APL : Above than the permissible limit.

NAPL : None above than the permissible limit.

a : Significant increase of migration of metals under sunlight in comparison with the migrations obtained in the hot air oven at the same temperature.

: Values are mean ±SE of four samples, $p < 0.05$ was considered to be significant(Student's 't' test).

Table - 20: Influence of Sunlight on the migration of heavy metals (APL, in ppm) from lunch boxes of bread 'B'.

Extractants	8 hours in hot air oven at 40-45°C	8 hours in sunlight at 40-45°C
Distilled water	Cd(3.05±0.30) Zn(4.80±0.24)	Cd(4.90±0.42)[a] Zn(5.24±0.48)
8% Ethanol	Zn(4.73±0.22)	Zn(5.08±0.32)
3% Acetic Acid	Cd(3.00±0.30) Zn(5.05±0.25	Cd(4.26±0.32)[a] Zn(6.28±0.42)[a] Cr(1.46±0.08)
5% Acetic Acid	Cd(4.02±0.38) Zn(6.03±0.28) Cr(1.42±0.06)	Cd(5.32±0.22)[a] Zn(7.90±0.24)[a] Cr(1.40±0.06)
5% Sodium Carbonate	Cd(3.20±0.30) Zn(5.93±0.22)	Cd(5.20±0.24)[a] Zn(7.99±0.23)[a]
0.9% Sodium Chloride	Cd(3.80±0.30)	Cd(4.68±0.20)[a]

Permissible limit (PL) : Concentrations of Cr, Cu, Mn, Zn & Fe in the plastic extracts should not exceed 1 ppm and that of Cd should not exceed 0.1 ppm.

APL : Above than the permissible limit.

NAPL : None above than the permissible limit.

a : Significant increase of migration of metals under sunlight in comparison with the migrations obtained in the hot air oven at the same temperature.

: Values are mean ±SE of four samples, $p < 0.05$ was considered to be significant(Student's 't' test).

Table - 21: Influence of sunlight on the migration of heavy metals (APL, in ppm) from blood bags of Brand 'A'.

Extractants	8 hours in hot air oven at 40-45°C	8 hours in sunlight at 40-45°C
Distilled water	NAPL	Pb(1.20±0.04)
8% Ethanol	NAPL	NAPL
3% Acetic Acid	Zn(1.42±0.08)	Pb(1.36±0.07) Zn(2.50±0.06)[a]
5% Acetic Acid	Pb(1.20±0.02) Zn(1.73±0.03)	Pb(1.50±0.08) Zn(2.70±0.06)[a]
5% Sodium Carbonate	Zn(1.42±0.03)	Pb(1.50±0.03) Zn(2.60±0.06)[a]
0.9% Sodium Chloride	NAPL	Pb(2.42±0.04)

Permissible limit (PL) : Concentrations of Cr, Cu, Mn, Zn & Pb in the plastic extracts should not exceed 1 ppm and that of Cd should not exceed 0.1 ppm.

APL : Above than the permissible limit.

NAPL : None above than the permissible limit.

a : Significant increase of migration of metals under sunlight in comparison with the migrations obtained in the hot air oven at the same temperature.

: Values are mean ±SE of four samples, $p < 0.05$ was considered to be significant(Student's 't' test).

Table - 22: Influence of pH and temperature on the migration of heavy metals (APL, in ppm) from freeze bottles of Brand 'A'.

Extractants / Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4)	5% Acetic Acid (pH = 2.5)	5% Sodium Carbonate (pH = 10)
40°C for 8 hrs.	NAPL	NAPL	NAPL	NAPL
40°C for 24 hrs.	NAPL	Cd(0.20±0.01)	Cd(0.68±0.03)[b]	Cd(0.75±0.04)[b]
60°C for 2 hrs.	NAPL	Cd(0.35±0.02)	Cd(0.72±0.09)[b]	Cd(0.80±0.02)[b]
60°C for 10 days	NAPL	Cd(0.80±0.06)	Cd(1.00±0.09)	Cd(1.20±0.08)[b]

Permissible limit (PL) : Cr, Cu, Pb, Mn & Zn should not be more than 1 ppm and Cd should not be more than 0.1 ppm.

APL : Above than the permissible limit.

NAPL : None above than the permissible limit.

b : Significant increased leaching of metals in comparison with 3% acetic acid.

: Values in the parenthesis are the average of four samples ±SE, $p < 0.05$ was considered to be significant (Student's 't' test).

Table - 23: Influence of pH and temperature on the migration of heavy metals (APL, in ppm) from freeze bottles of Brand 'B'.

Extractants / Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4)	5% Acetic Acid (pH = 2.5)	5% Sodium Carbonate (pH = 10)
40°C for 8 hrs.	NAPL	NAPL	NAPL	NAPL
40°C for 24 hrs.	NAPL	Zn(1.2±0.02)	Zn(1.4±0.06)	Pb(1.8±0.06)
60°C for 2 hrs.	NAPL	NAPL	Zn(1.3±0.06)	Pb(1.6±0.04)
60°C for 10 days	Pb(1.8±0.06) Zn(1.1±0.04)	Pb(2.4±0.02)[a] Zn(1.4±0.06)	Pb(4.9±0.00)[b] Zn(2.3±0.04)[b]	Pb(2.5±0.08)[a] Cd(1.0±0.13)

Permissible limit (PL) : Pb, Cr, Cu, Mn & Zn should not exceed 1 ppm & Cd should not exceed 0.1 ppm.

APL : Above than the permissible limit.

NAPL : None above than the permissible limit.

a : Significant increased leaching of metals with respect to distilled water.

b : Significant increased leaching of metals with respect to 3% Acetic Acid.

: Values in the parenthesis are the average of four samples ±SE, $p < 0.05$ was considered to be significant (Student's 't' test).

Table - 24: Influence of pH and temperature on the migration of heavy metals (APL, in ppm) from water tumblers of Brand 'A'.

Extractants / Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4)	5% Acetic Acid (pH = 2.5)	5% Sodium Carbonate (pH = 10)
40°C for 8 hrs.	Cd(0.25±0.00)	Cd(0.30±0.00)	Cd(1.00±0.05)[b]	Cd(0.50±0.12)
40°C for 24 hrs.	Cd(0.30±0.04)	Cd(0.40±0.04) Cr(1.60±0.04)	Cd(1.0±0.12)[b] Cr(2.1±0.06)[b]	Cd(0.80±0.08)[a] Cr(1.20±0.10)
60°C for 2 hrs.	Cd(0.40±0.08)	Cd(0.70±0.09)[a]	Cd(1.2±0.08)[b]	Cd(0.40±0.10)
60°C for 10 days.	Cd(0.46±0.02) Cu(1.4±0.04)	Cd(1.74±0.02)[a] Cu(2.00±0.00) Cr(3.13±0.12)	Cd(2.00±0.14)[a] Cu(3.00±0.08)[b] Cr(3.10±0.08)[a]	Cd(1.40±0.06)[a] Cr(1.60±0.04)

Permissible limit (PL) : Cu, Cr, Mn, Zn & Pb not more than 1 ppm and Cd should not exceed 0.1 ppm.

APL : Above than the permissible limit.

NAPL : None above than the permissible limit.

a : Significant increase of leaching of metals with respect of distilled water.

b : Significant increase of leaching of metals with respect to 3% Acetic Acid.

: Values in parenthesis are the average of four samples ±SE, $p < 0.05$ was considered to be significant (Student's 't' test).

Table - 25: Influence of pH and temperature on the migration of heavy metals (APL, in ppm) from water tumblers of Brand 'B'.

Extractants / Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4)	5% Acetic Acid (pH = 2.5)	5% Sodium Carbonate (pH = 10)
40°C for 8 hrs.	NAPL	NAPL	NAPL	NAPL
40°C for 24 hrs.	NAPL	Cd(0.18±0.01)	Cd(0.26±0.08)[b]	Cd(0.28±0.03)[b]
60°C for 2 hrs.	NAPL	Cd(0.12±0.00)	Cd(0.23±0.04)[b]	Cd(0.24±0.06)[b]
60°C for 10 days	NAPL	Cd(0.24±0.06)	Cd(0.40±0.09)[b]	Cd(0.38±0.08)[b]

Permissible limit (PL) : Cu, Cr, Zn, Mn & Pb should not exceed 1 ppm and Cd should not exceed 0.1 ppm.

APL : Above than the permissible limit.

NAPL : None above than the permissible limit.

b : Significant increase of leaching of metals with respect to 3% Acetic Acid.

: Values in parenthesis are the average of four samples ±SE, $p < 0.05$ was considered to be significant (Student's 't' test).

Table - 26: Influence of pH and temperature on the migration of heavy metals (APL, in ppm) from lunch boxes of brand'A'.

Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4)	5% Acetic Acid (pH = 2.5)	5% Na_2CO_3 (pH = 10)
40°C for 8 hours	Cd(0.20 ± 0.04) Pb(1.20 ± 0.04)	Cd(0.60 ± 0.04)[a] Pb(1.40 ± 0.05)	Cd(0.7 ± 0.04)[a] Pb(1.4 ± 0.06)	Cd(0.4 ± 0.04)[a]
40°C for 24 hours	Cd(0.30 ± 0.05) Pb(1.20 ± 0.06)	Cd(0.60 ± 0.04)[a] Pb(1.40 ± 0.07)	Cd(0.8 ± 0.04)[a] Pb(1.4 ± 0.07)	Cd(0.8 ± 0.05)[a]
60°C for 2 hours	Cd(0.40 ± 0.02) Cu(1.50 ± 0.01)	Cd(0.60 ± 0.06)[a] Cu(1.80 ± 0.03)	Cd(0.6 ± 0.06)[a] Cu(1.8 ± 0.03)	Cd(0.5 ± 0.04)
60°C for 10 days	Cd(0.60 ± 0.08) Cu(1.60 ± 0.08) Pb(1.20 ± 0.03)	Cd(0.80 ± 0.04)[a] Cu(1.90 ± 0.06) Pb(1.60 ± 0.08)	Cd(1.7 ± 0.10)[b] Cu(2.4 ± 0.14)[b] Pb(1.9 ± 0.04)[a]	Cd(1.8 ± 0.06)[b] Pb(1.6 ± 0.04)

Permissible limit (PL): Cu, Cr, Zn, Mn & Pb should not exceed 1 ppm and Cd should not exceed 0.1 ppm.

APL : Above than the permissible limit.

a : Significant increased leaching of metals with respect to distilled water.

b : Significant increased leaching of metals with respect to 3% acetic acid.

Values in parenthesis are the average of four samples ± S.E., $p<0.05$ was considered to be significant (studuent's test).

Table - 27 : Influence of pH and temperature on the migration of heavy metals (APL, in ppm) from lunch boxes of brand-'B'.

Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% Na_2CO_3 (pH = 10)
40°C for 8 hours	NAPL	Cd(1.43 ± 0.10)	Cd(1.41 ± 0.20)	Cd (1.89 ± 0.16)
40°C for 24 hours	Cd (2.80 ± 0.24) Zn (4.64 ± 0.38)	Cd(3.50 ± 0.30) Zn(5.56 ± 0.24) Cr(1.20 ± 0.08)	Cd(4.72 ± 0.42) Zn(6.08 ± 0.30) Cr(1.40 ± 0.24)	Cd(3.92 ± 0.38) Zn(5.82 ± 0.29)
60°C for 2 hours	Cd(3.03 ± 0.30) Zn(4.88 ± 0.29)	Cd(3.83 ± 0.24) Zn(5.20 ± 0.30)	Cd(4.13 ± 0.30) Zn(5.87 ± 0.24)	Cd(4.08 ± 0.26) Zn(5.00 ± 0.84)
60°C for 10 days	Cd(5.40 ± 0.80) Zn(5.24 ± 0.92)	Cd(6.90 ± 0.34) Zn(7.63 ± 0.48) Cr(1.46 ± 0.19)	Cd(7.56 ± 0.88) Zn(8.49 ± 0.68) Cr(1.80 ± 0.48)	Cd(7.46 ± 0.30) Zn(7.60 ± 0.49)

Permissible limit: Cr, Cu, Pb, Mn & Zn should not be more than 1 ppm and Cd should not be more than 0.1 ppm.

NAPL : None above than the permissible limit.

APL : Above than the permissible limit.

: Values in the parenthesis are the average of four samples ±S.E., $p < 0.05$ was considered to be significant (studuent's t test).

Table - 28: Influence of pH and temperature on the migration of heavy metals (APL, in ppm) from blood bags of brand-A.

Temperature	Distilled water (pH=6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% Na_2CO_3 (pH = 10)
40°C for 8 hours	NAPL	NAPL	Pb (1.2 ± 0.04)	NAPL
40°C for 24 hours	NAPL	Pb (1.20 ± 0.02)	Pb (1.30 ± 0.06)	Pb (1.30 ± 0.04)
60°C for 2 hours	NAPL	Pb (1.20 ± 0.02)	Pb (1.40 ± 0.02)	Pb (1.25 ± 0.03)
60°C for 10 days	Pb (1.2 ± 0.05)	Pb (1.40 ± 0.09) Zn (2.90 ± 0.02)	Pb (1.60 ± 0.12)[a] Zn (2.90 ± 0.09)	Pb (1.60 ± 0.06)[a] Zn (2.70 ± 0.65)

Permissible limit: Pb, Mn, Cr, & Zn should not exceed 1 ppm and Cd should not exceed 0.1 ppm.

APL : Above than the permissible limit.

NAPL : None above than the permissible limit.

a : Significant increased leaching of metals with respect to distilled water.

Values in parenthesis are the average of four samples ± S.E., $p < 0.05$ was considered to be significant (student's 't' test)

Table -29: Influence of pH and temperature on the migration of U.V. absorbing materials (in O.D.) from freeze bottles of brand 'A'

EXTRACTANTS Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% $Na_2 CO_3$ (pH = 10)
40°C for 8 hours	0.18	0.27	0.36*	0.38*
40°C for 24 hours	0.28	0.40*	0.45*	0.46*
60°C for 2 hours	0.30	0.38*	0.41*	0.49*
60°C for 10 days	0.51*	2.54*	2.72*	2.69*

Permissible limits : not more than 0.3 O.D.

* : Above than the permissible limits.

: Values are the mean of four samples.

Table - 30: Influence of pH and temperature on the migration of U.V. absorbing materials (in O.D.) from freeze bottles of brand 'B'.

EXTRACTANTS Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% $Na_2 CO_3$ (pH = 10)
40°C for 8 hours	0.00	0.08	0.10	0.10
40°C for 24 hours	0.08	0.15	0.27	0.28
60°C for 2 hours	0.06	0.20	0.28	0.30
60°C for 10 days	0.14	0.24	0.32*	0.36*

Permissible limit: not more than 0.3 O.D.

* : Above than the permissible limit.

: Values are the mean of four samples.

Table 31: Influence of pH and temperature on the migration of U.V. absorbing materials (in O.D.) from water tumblers of brand 'A'.

EXTRACTANTS Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% $Na_2 CO_3$ (pH = 10)
40°C for 8 hours	0.30	0.45*	0.49*	0.49*
40°C for 24 hours	0.40*	0.52*	0.54*	0.59*
60°C for 2 hours	0.31*	0.46*	0.50*	0.53*
60°C for 10 days	0.59*	1.45*	2.00*	2.73*

Permissible limits : not more than 0.3 O.D.

* : Above than the permissible limits.

: Values mean of four samples.

Table - 32: Influence of pH and temperature on the migration of U.V. absorbing materials (in O.D.) from water tumblers of brand 'B'.

EXTRACTANTS Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% $Na_2 CO_3$ (pH = 10)
40°C for 8 hours	0.06	0.26	0.39*	0.34*
40°C for 24 hours	0.18	0.38*	0.50*	0.48*
60°C for 2 hours	0.16	0.28	0.39*	0.37*
60°C for 10 days	0.28	0.56*	0.60*	0.54*

Permissible limits : not more than 0.3 O.D.

* : Above than the permissible limits.

: Values are mean of four samples.

Table - 33: Influence of pH and temperature on the migration of U.V. absorbing materials (in O.D.) from lunch boxes of brand 'A'.

EXTRACTANTS Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% $Na_2 CO_3$ (pH = 10)
40°C for 8 hours	0.12	0.23	0.28	0.28
40°C for 24 hours	0.19	0.30	0.34*	0.31*
60°C for 2 hours	0.10	0.19	0.24	0.18
60°C for 10 days	1.23*	2.08*	2.39*	2.05*

Permissible limit : not more than 0.3 O.D.

* : Above than the permissible limit.

: Values are the mean of four samples.

Table - 34: Influence of pH and temperature on the migration of U.V. absorbing materials (in O.D.) from lunch boxes of brand 'B'.

EXTRACTANTS / Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% Na_2CO_3 (pH = 10)
40°C for 8 hours	0.12	0.68*	1.06*	1.20*
40°C for 24 hours	0.16	0.78*	1.40*	1.48*
60°C for 2 hours	0.20	0.84*	1.84*	2.00*
60°C for 10 days	0.26	0.98*	2.31*	2.42*

Permissible limit : not more than 0.3 O.D.

* : Above than the permissible limit.

: Values are the mean of four samples.

Table - 35: Influence of pH and temperature on the migration of U.V. absorbing materials (in O.D.) from blood bags of brand 'A'.

EXTRACTANTS Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% Na_2CO_3 (pH = 10)
40°C for 8 hours	0.09	0.20	0.24	0.26
40°C for 24 hours	0.12	0.26	0.29	0.32*
60°C for 2 hours	0.10	0.21	0.26	0.31*
60°C for 10 days	0.20	0.30	0.32*	0.36*

: Permissible limit : not more than 0.3 O.D.

* : Above than the permissible limit.

: Values are the mean of four samples.

Table - 36: Influence of pH and temperature on the migration of U.V. absorbing materials (in O.D.) from blood bags of brand 'B'.

EXTRACTANTS / Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% Sodium Carbonate (pH = 10)
40°C for 8 hours	0.09	0.18	0.20	0.23
40°C for 24 hours	0.12	0.19	0.24	0.24
60°C for 2 hours	0.16	0.20	0.26	0.27
60°C for 10 days	0.19	0.24	0.28	0.30

: Permissible limit : not more than 0.3 O.D.

: Values are the mean of four samples.

Table - 37: Influence of pH and temperature on the global migration (mg/100 ml of extract) from freeze bottles of brand-A.

EXTRACTANTS Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% sodium carbonate (pH = 10)
40°C for 8 hours	(2.14 ± 0.02)	(3.60 ± 0.03)^{a}	(4.80 ± 0.25)^{b}	(5.90 ± 0.41)^{*b}
40°C for 24 hours	(2.81 ± 0.01)	(3.80 ± 0.15)^{a}	(5.83 ± 0.12)^{*b}	(8.93 ± 0.15)^{*b}
60°C for 2 hours	(1.43 ± 0.03)	(3.50 ± 0.12)^{a}	(5.50 ± 0.11)^{*b}	(8.80 ± 0.10)^{*b}
60°C for 10 days	(3.03 ± 0.09)	(7.90 ± 0.38)^{*a}	(14.87 ± 0.24)^{*b}	(23.43 ± 0.75)^{*b}

Permissible limit (PL): Global migration should not be more than 5 mg/100 ml of extract.

* : Above than the permissible limit.

a : significant increase of global migration in comparison with distilled water.

b : significant increase of global migration in comparison with 3% acetic acid.

: Values are mean ± S.E. of four samples, $p < 0.05$ was considered to be significant (Student's 't' test).

Table - 38 : Influence of pH and temperature on the global migration (mg/100 ml of extract) from freeze bottles of brand-B.

EXTRACTANTS Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% sodium carbonate (pH = 10)
40°C for 8 hours	(2.10 ± 0.06)	(4.80 ± 0.12)^{a}	(5.30 ± 0.14)^{*b}	(5.33 ± 0.18)^{*b}
40°C for 24 hours	(2.40 ± 0.15)	(5.60 ± 0.21)^{*a}	(8.07 ± 0.09)^{*b}	(8.46 ± 0.24)^{*b}
60°C for 2 hours	(2.63 ± 0.09)	(4.80 ± 0.26)^{a}	(5.23 ± 0.12)^{*a}	(6.57 ± 0.20)^{*b}
60°C for 10 days	(5.33 ± 0.20)*	(11.40 ± 0.25)^{*a}	(12.63 ± 0.24)^{*b}	(13.07 ± 0.52)^{*b}

Permissible limit (PL): Global migration should not be more than 5.0 mg/100 ml of extract.

* : Above that the permissible limit.

a : Significant increase of global migration in comparison with distilled water.

b : Significant increase of global migration in comparison with 3% acetic acid.

: Values are mean ± S.E. of four samples, $p < 0.05$ was considered to be significant (Student's 't' test).

Table - 39 : Influence of pH and temperature on the global migration (mg/100 ml of extract) from water tumblers of brand-A.

EXTRACTANTS Temperature	Distilled water (pH =6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% sodium carbonate (pH = 10)
40°C for 8 hours	(1.30 ± 0.13)	(3.40 ± 0.16)^{a}	(5.40 ± 0.14)^{*b}	(7.20 ± 0.13)^{*b}
40°C for 24 hours	(4.20 ± 0.12)	(4.80 ± 0.40)	(7.20 ± 0.40)^{*b}	(12.33 ± 0.44)^{*b}
60°C for 2 hours	(5.86 ± 0.41)*	(6.08 ± 0.17)*	(8.93 ± 0.56)^{*b}	(12.53 ± 0.52)^{*b}
60°C for 10 days	(7.00 ± 0.25)*	(11.10 ± 0.32)^{*a}	(15.90 ± 0.44)^{*b}	(33.77 ± 0.64)^{*b}

Permissible limit (PL): Global migration should not be more than 5.0 mg/100 ml of extract.

* : Above than the permissible limit.

a : Significant increase of global migration in comparison with distilled water.

b : Significant increase of global migration in comparison with 3% Acetic Acid.

: Values are mean ± S.E. of four samples, $p < 0.05$ was considered to be significant (Student's 't' test).

Table - 40: Influence of pH and temperature on the global migration (mg/100 ml of extract) from water tumblers of brand-B.

EXTRACTANTS Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% Sodium carbonate (pH = 10)
40°C for 8 hours	(2.90 ± 0.09)	(7.40 ± 0.13)*a	(7.99 ± 0.13)*a	(7.90 ± 0.24)*a
40°C for 24 hours	(3.90 ± 0.07)	(9.80 ± 0.35)*a	(10.70 ± 0.36)*a	(10.20 ± 0.15)*a
60°C for 2 hours	(2.50 ± 0.15)	(6.20 ± 0.12)*a	(9.40 ± 0.29)*b	(8.90 ± 0.12)*b
60°C for 10 days	(8.33 ± 0.58)*	(43.20 ± 0.53)*a	(52.30 ± 0.68)*b	(42.36 ± 1.72)*a

Permissible limit (PL): Global migration should not be more than 5.0 mg/100 ml of extract.

* : Above than the permissible limit.

a : Significant increase of global migration in comparison with distilled water.

b : Significant increase of global migration in comparison with 3% acetic acid.

: Values are mean ± S.E. of four samples, $p < 0.05$ was considered to be significant (Student's 't' test).

Table 41 : Influence of pH and temperature on the global migration (mg/100 ml of extract) from lunch boxes of brand-A.

EXTRACTANTS / Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% Sodium carbonate (pH = 10)
40°C for 8 hours	(1.84 ± 0.02)	(2.60 ± 0.06)^{a}	(3.30 ± 0.15)^{b}	(5.63 ± 0.12)^{*b}
40°C for 24 hours	(2.50 ± 0.17)	(5.63 ± 0.09)^{*a}	(6.53 ± 0.09)^{*b}	(7.40 ± 0.10)^{*b}
60°C for 2 hours	(2.50 ± 0.06)	(3.40 ± 0.12)^{a}	(4.70 ± 0.09)^{b}	(5.46 ± 0.18)^{*b}
60°C for 10 days	(5.63 ± 0.18)*	(9.27 ± 0.18)^{*a}	(17.33 ± 0.24)^{*b}	(30.47 ± 0.61)^{*b}

Permissible limit (PL): Global migration should not be more than 5.0 mg/100 ml of extract.

* : Above than the permissible limit.

a : Significant increase of global migration in comparison with distilled water.

b : Significant increase of global migration in comparison with 3% acetic acid.

: Values are mean ± S.E. of four samples, $p<0.05$ was considered to be significant (Student's 't' test).

Table - 42: Influence of pH and temperature on the global migration (mg/100 ml of extract) from lunch boxes of brand-B.

EXTRACTANTS Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% sodium carbonate (pH = 10)
40°C for 8 hours	(3.50 ± 0.03)	(5.30 ± 0.08)*a	(6.25 ± 0.12)*a	(6.80 ± 0.10)*b
40°C for 24 hours	(4.25 ± 0.06)	(6.45 ± 0.14)*a	(8.20 ± 0.13)*b	(9.00 ± 0.14)*b
60°C for 2 hours	(4.00 ± 0.08)	(6.20 ± 0.10)*a	(8.00 ± 0.14)*b	(8.46 ± 0.12)*b
60°C for 10 days	(5.90 ± 0.08)*	(9.20 ± 0.18)*a	(10.45 ± 0.16)*b	(12.48 ± 0.19)*b

Permissible limit (PL) : Global migration should not be more than 5.0 mg/100 ml of extract.

* : Above than the permissible limit.

a : Significant increase of global migration in comparison with distilled water.

b : Significant increase of global migration in comparison with 3% acetic acid.

: Values are mean ± S.E. of four samples, $p < 0.05$ was considered to be significant (Student's 't' test).

Table 43 : Influence of pH and temperature on the global migration (mg/100 ml of extract) from blood bags of brand-A.

EXTRACTANTS Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% sodium carbonate (pH = 10)
40 °C for 8 hours	(1.20 ± 0.06)	(1.90 ± 0.08)[a]	(3.40 ± 0.04)[b]	(4.50 ± 0.12)[b]
40 °C for 24 hours	(1.20 ± 0.06)	(2.40 ± 0.07)[a]	(3.50 ± 0.07)[b]	(4.90 ± 0.03)[b]
60 °C for 2 hours	(1.00 ± 0.02)	(2.10 ± 0.04)[a]	(2.50 ± 0.03)[b]	(3.90 ± 0.08)[b]
60 °C for 10 days	(3.80 ± 0.00)	(4.70 ± 0.21)[a]	(5.80 ± 0.08)[*b]	(6.50 ± 0.18)[*b]

Permissible limit (PL) : Global migration should not be more than 5.0 mg/100 ml of extract.

* : Above than the permissible limit.

a : Significant increase of global migration in comparison with distilled water.

b : Significant increase of global migration in comparison with 3% acetic acid.

: Values are mean ± S.E. of four samples, $p \leq 0.05$ was considered to be significant (Student's 't' test).

Table - 44: Influence of pH and temperature on the global migration (mg/100 ml of extract) from blood bags of brand-B.

EXTRACTANTS Temperature	Distilled water (pH = 6.5)	3% Acetic Acid (pH = 4.0)	5% Acetic Acid (pH = 2.5)	5% sodium carbonate (pH = 10)
40°C for 8 hours	(1.80 ± 0.08)	(2.68 ± 0.04)[a]	(3.90 ± 0.10)[b]	(3.95 ± 0.08)[b]
40°C for 24 hours	(1.90 ± 0.06)	(2.90 ± 0.08)[a]	(3.98 ± 0.12)[b]	(3.99 ± 0.06)[b]
60°C for 2 hours	(1.20 ± 0.06)	(2.60 ± 0.03)[a]	(3.74 ± 0.08)[b]	(3.85 ± 0.06)[b]
60°C for 10 days	(2.20 ± 0.10)	(3.45 ± 0.09)[a]	(4.05 ± 0.16)[b]	(4.90 ± 0.09)[b]

Permissible limit (PL) : Global migration should not be more than 5.0 mg/100 ml extract.

* : Above than the permissible limit.

a : Significant increase of global migration in comparison with distilled water.

b : Significant increase of global migration in comparison with 3% acetic acid.

: Values are mean ± S.E. of four samples, $p < 0.05$ was considered to be significant (Student's 't' test).

CHAPTER-3: EFFECT OF AGE & SEX ON THE TOXICITY OF DIBUTYLTIN DILAURATE (DBTL) - A LEACHABLE PLASTIC ADDITIVE: NEUROBEHAVIORAL AND BIOCHEMICAL EFFECTS.

INTRODUCTION:

Dibutyltin dilaurate is an organotin compound, commonly used as PVC stabilizer, as biocides and as catalytic agents. The workers engaged in the production and processing of plastics and handling plastic utensils are exposed to varying amount of the organotin compounds. The general population including the pregnant mothers are also exposed to certain levels of organotin compounds for prolonged periods of time due to their leaching from finished plastics and contamination of the food chain as a result of their use as fungicides. The widespread use of organotin compounds and reports of their migration from PVC food containers into liquid food and oil and their entry into the biological systems has aroused a great concern over their toxicological potential.

So far extensive studies have been conducted on the toxicity produced by lower homologues of organotin compounds. However, adequate information about the toxicity produced by higher homologues of organotin compounds are not known. In humans, exposure to organotin can occur to all ages including the neonates, through plastic feeding bottles, pacifier, toys, transfusion pouches etc., it was thought worthwhile to study the role of age and sex on certain behavioral and biochemical parameters to ascertain neurotoxicity of higher homologues of organotin compounds, since brain is reported to be the target organ for the organotin toxicity. The ability of the body to metabolize xenobiotic varies with age and sex, it is reasonable to expect that organotin compounds may exhibit an age and sex related toxicity. Experimental studies have also indicated variations in the toxicity of chemicals with age and sex of the experimental animals (185-188).

To investigate this, the candidate has studied in depth the biochemical and behavioral effects of dibutyltin dilaurate a higher homologue of organotin compounds, using albino rats of different age i.e., weanling (4 week), Juvenile (8 week) and Adult (24 week) and sex (Male & Female) as the experimental model.

Materials & Methods:

Equipments Used:

1. Kontron Spectrofluorometer, SFM-23/B.
2. Sorvall RC-5B, Superspeed Cold Centrifuge.
3. Digital Photo-Actometer, Techno India.
4. Cook's Pole Climbing Response Apparatus, Techno, India.
5. Remi T-8 Table Top Centrifuge.
6. Homogenizer, Arthurtho.

ANIMALS & TREATMENT:

Weanling, Juvenile and Adult rats of both sex from ITRC animal breeding colony maintained on standard pellet diet (Hindustan Lever, Laboratory Animals Feed, India) and water ad libitum were used. The age and body weight range of the male rats were: Weanling (about 4 weeks old, 35-40 gm), Juvenile (about 8 weeks old, 115-125 gm) and adults (about 24 weeks old, 340-355 gm). The female rats were of the same age as the respective group of male animals and their body weights were: Weanling (30-35 gm), Juvenile (95-110 gm) and adults (285-300 gm). The rats were randomly assigned into four groups of 25 animals each and given 0, 20, 40 or 80 mg/kg DBTL, diluted in groundnut oil, orally for 3 consecutive days. Eight animals from each group were used for

locomotor activity and learning ability and another set of six animals from each group were used for the estimation of biogenic amines.

BEHAVIORAL TESTS:

Following behavioral tests were performed on the treated and control animals.

SPONTANEOUS LOCOMOTOR ACTIVITY:

Twenty four hours after the last treatment, 8 rats each from the control and treated groups were removed and placed individually in a photoactometer (Techno, India). The spontaneous locomotor activity was recorded as counts per minute for a period of 10 minutes. The drug induced motor activity was recorded next day in these animals. The animals were exposed to amphetamine, 2.5 mg/kg body weight and after 10 minutes placed individually in a photoactometer and the drug induced motor activity was recorded as counts per minute for a period of 10 minutes. All the studies were conducted between 10.00 and 18.00 hours. The box was wiped clean before placing each rat for the testing.

LEARNING ABILITY:

On the second day after the treatment, rats were removed from the home cage and placed in the chamber of the Cook's Pole climbing Apparatus (Techno, India).

The conditioned stimuli were the sound of a buzzer and turning of light in the compartment occupied by the rat. If the rat did not climb the pole within 10 seconds the unconditioned stimulus (an electric shock of 400 v and 0.2 mA) was delivered through the grid-floor of the box.

An avoidance trial included a maximum of 10 seconds of conditioned stimulus (CS) alone followed by 10 seconds of both CS and

Unconditioned stimulus (U.S.) and 40 seconds intertrial interval (ITI). The unconditioned stimuli was terminated as soon as the rat avoided the foot shock by climbing the pole. The number of times the rat climbed the pole during the conditioned stimulus period was considered to reflect learning. The index for the evaluation of the acquisition process was the response rate, (number of times rat climbed the pole or % avoidance rate) of the arrival.

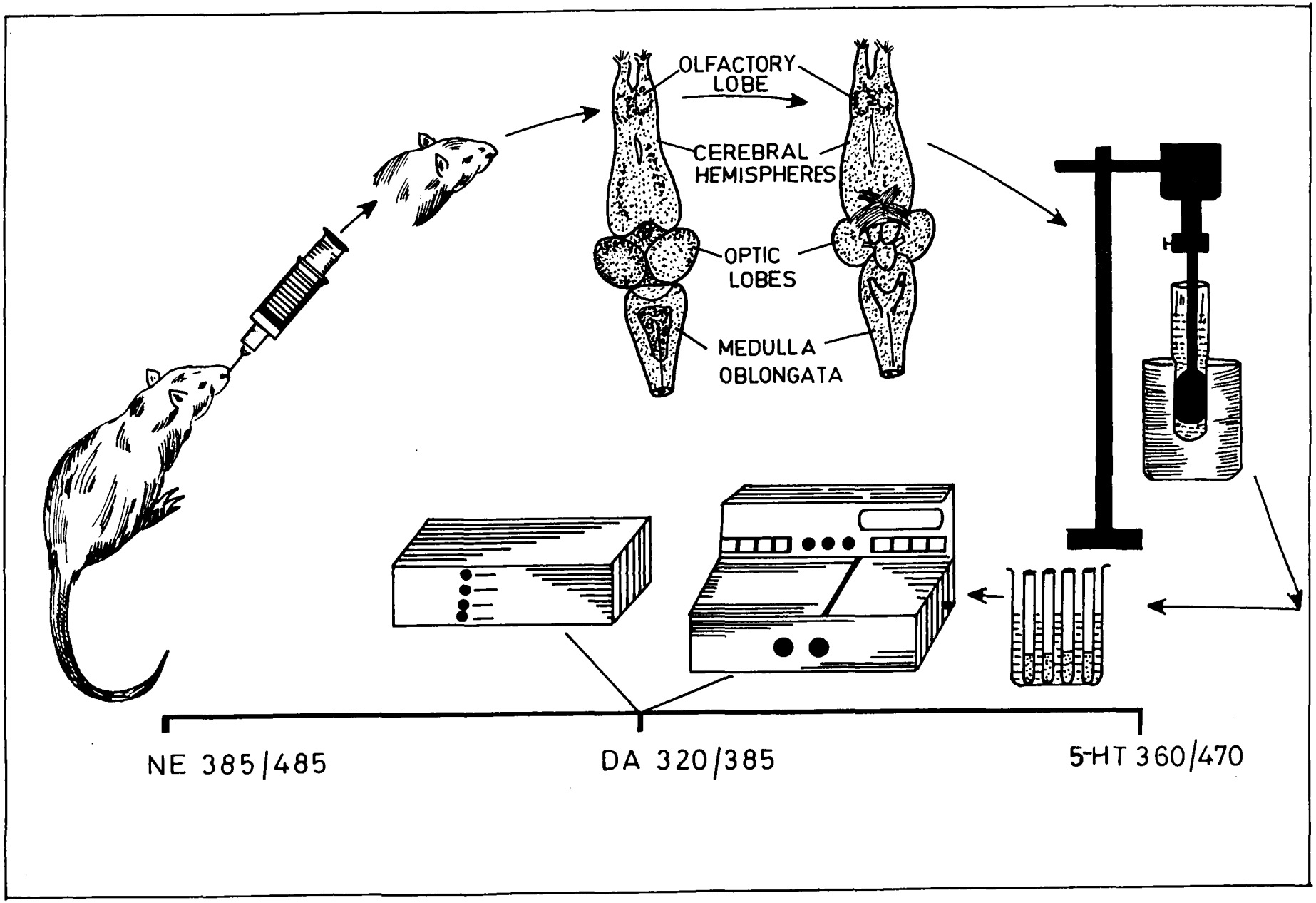
ESTIMATION OF NOREPINEPHRINE (NE) DOPAMINE (DA) AND 5-Hydroxy-TRYPTAMINE IN THE WHOLE BRAIN HOMOGENATE AND IN DIFFERENT BRAIN AREAS:

Eight animals from each group were sacrificed after three days exposure to DBTL by decapitation. Brains were removed, dissected into various regions (Hypothalamus, cerebellum, pons medulla and frontal cortex), weighed and homogenized in chilled butanol to which a desired amount of 0.01 N HCL was added. The homogenates was centrifuged at 1500 rpm for 10 minutes to sediment the tissue debris. The clear supernatant (2 ml) was aspirated out into a centrifuge tube to which 1.5 ml of 0.1 N phosphate buffer was added. The contents were recentrifuged at 3000 rpm for 20 minutes to extract the dopamine and norepinephrine. Another aliquot of the clear supernatent (2 ml) was aspirated out in a centrifuge tube containing 5 ml heptane and 0.5 ml of 0.1 N HCL for the determination of 5-HT. The contents were centrifuged at 3000 rpm for 20 minutes. These aliquots were further oxidised into fluorophores by the addition of Ortho-phthaldialdehyde. NE fluorescence were read at 385/485, DA at 320/385 and 5-HT at 360/470, using a Kontron-Spectrofluorometer, SFM-23/B. Slopes were calculated from the standards of the different biogenic amines and the concentrations were expressed

as ug/gm fresh tissue weight by dividing the fluorescence reading of standards by slope reading and the tissue weight. The method of Jacobwitz and Richardson (189) was adopted for the estimation of biogenic amines.

STATISTICAL ANALYSIS:

Data was evaluated by the student's 't' test (183). A value of $p < 0.05$ was considered to be significant.


OLFACTORY LOBE
CEREBRAL HEMISPHERES
OPTIC LOBES
MEDULLA OBLONGATA
NE 385/485
DA 320/385
5-HT 360/470

RESULTS

Gross appearance:

The animals received DBTL (20, 40, or 80 mg/kg) were found to be lethargic, dull, weak throughout the experimental periods as compared to the controls. Swelling and reddening around the mouth area associated with brown pigmentation on the central body surface and hindlimb weakness was also observed in the group of animals exposed to two higher doses (40 and 80 mg/kg) of DBTL.

Body weight:

Pattern of loss in body weight of different groups of animals exposed to various doses of DBTL (20, 40 or 80 mg/kg) is shown in Tables (45-47). A gradual loss in the body weight of DBTL exposed rats was observed in weanling, Juvenile and adult rats in comparison to the age matched controls in a dose dependent manner, which was significant in the animals exposed to the two higher doses of DBTL. Juvenile rats of both sex were found to exhibit maximum decrease in their body weight in comparison to the other group of animals (Figure-46). However, DBTL had no significant effect on the wet weight of the brain (total or relative to body weight) in all the groups of animals except the animals exposed to 80 mg/kg.

Mortality:

The percent mortality in the animals of different age (4,8,24 weeks) and sex (males & females) treated with DBTL is shown in Table (48). As evident from the table, no mortality was recorded in the groups of animals served as controls. The animals exposed to 20 mg/kg DBTL showed no mortality either in the males or females of weanling group,

while the adult rats showed 10% mortality in males and 20% mortality in females. When the exposure of DBTL was increased to 40 mg/kg, the weanling and juvenile rats showed 10% and 20% mortality in males and females respectively, whereas the adult rats showed 40% and 60% mortality in males and females respectively. When the exposure of DBTL was further increased to 80 mg/kg, an increased rate of mortality was noticed in all the groups of animals. The animals of weanling group showed 20% and 30% mortality in males and females respectively while the animals of juvenile group showed 30% mortality in both sexes and the adult animals showed 60% in males and 90% in females.

Our results suggest that the mortality was dose dependent and the female rats of all ages were found to be more prone to the exposure of DBTL.

Spontaneous and Drug induced motor activity:

Effect of various doses of DBTL on the spontaneous and drug induced motor activity in weanling, juvenile and adult male and female albino rats is shown in Figures (37-39).

As apparent from Figure (37), the weanling male and female rats exposed to DBTL showed a dose dependent decrease in the spontaneous and drug induced motor activity. In weanling male rats the percentage decrease in the spontaneous motor activity was 54, 65 and 71 at 20, 40 and 80 mg/kg dose of DBTL respectively. Whereas, in weanling female rats the percentage decrease in the spontaneous motor activity was 72, 80 and 81 at 20, 40 and 80 mg/kg dose of DBTL respectively. As a result of exposure to amphetamine to these animals, resulted an increase in the counts of motor activity and in male the percentage increase was 55.5, 67.3, 67.1 and 67 in the rats exposed to 0, 20, 40 and 80 mg/kg

DBTL respectively. Whereas, in females the percentage increase was 57.9, 65.4, 67.8, and 67.5 at 0, 20, 40 and 80 mg/kg DBTL respectively. The drug induced motor activity was also found to be affected as a result of exposure to DBTL. The percentage decrease in the males was 43.5, 57.6 and 62.9 while in females it was, 66.4, 75 and 75.5 in the rats exposed to 20, 40 and 80 mg/kg DBTL respectively.

Figure 38 shows the effect of various doses of DBTL on the spontaneous and drug induced motor activity in the juvenile rats. As apparent from the figure, the juvenile rats also showed a dose related decrease in the spontaneous and drug induced motor activity. The percentage decrease in the spontaneous motor activity in male rats was found to be 70.3, 77.4 and 80.8 while in female it was 82.4, 86 and 89 at 20, 40 and 80 mg/kg dose of DBTL respectively. The percentage decrease in the drug induced motor activity was 72.6, 73.9 and 76.3 in male and 79.9, 81.8 and 82.1 in female at 20, 40 and 80 mg/kg dose of DBTL respectively. Amphetamine increased the motor activity and was found to be 62.3%, 63.2%, 67.3 and 68.9% in male and 57.5%, 62.9%, 67.3% and 68.4% in juvenile female rats exposed to 0, 20, 40 and 80 mg/kg DBTL respectively.

Effect of various doses of DBTL on the spontaneous and drug induced motor activity in adult rats is shown in figure 39. As evident from the figure, adult male and female rats also showed a dose dependent decrease in the spontaneous and drug induced motor activity. The percentage decrease in the spontaneous motor activity was 60, 65.3 and 69.2 in male and 59.3, 71.3 and 77.6 in female adult rats at 20, 40 and 80 mg/kg dose of DBTL respectively. The percentage decrease in the drug induced motor activity was found to be 50.7, 53.9 and 57.3 in male rats and 49.6, 59.4 and 69.2 in female rats exposed to 20, 40 and 80 mg/kg

DBTL respectively. An increased rate of motor activity as a result of exposure of amphetamine was noticed and in male it was found to be 59.1, 66.8, 69.9 and 70.5 and 57.2, 65.4, 69.7 and 69.6 in female rats exposed to 0, 20, 40 and 80 mg/kg DBTL respectively.

Learning Ability:

Effect of various doses of DBTL on learning ability as assessed by CAR in weanling, juvenile and adult male and female albino rats is shown in figure 40-42. As evident from the figure (40), the weanling male rats showed 9%, 33.3% and 39.6% decrease in the 1st day conditioned avoidance response (CAR) and 7%, 19.5% and 29.9% in the 4th day CAR at 20, 40 and 80 mg/kg DBTL dose respectively. However, the percentage decrease in weanling female rats was 21.5, 92.6 and 132.1 in the 1st day CAR and 15.3, 28.0 and 36.8 in the 4th day CAR at 20, 40 and 80 mg/kg dose of DBTL respectively. Effect of DBTL exposure on CAR in the animals of juvenile group is shown in Fig. 41. As apparent from the figure the percent decrease in the 1st day CAR was 33.3, 45.8 and 50.0 in males and 42.6, 53.9 and 60.0 in females exposed to 20, 40 and 80 mg/kg DBTL dose respectively. A dose dependent decrease in the 4th day CAR was also noticed and was found to be 22.4, 28.1, 37.9 in males and 26.9, 37.4 and 45.2 in females exposed to 20, 40 and 80 mg/kg DBTL dose respectively. However, in adult groups the percentage decrease in the 1st day CAR was 16.3, 28.4 and 48.3 in males and 32.8, 45.0 and 48.3 in females exposed to 20, 40 and 80 mg/kg DBTL respectively (Fig.42). A dose dependent decrease in the 4th day CAR was also noticed and was found to be, 20.5%, 27.3% and 30.3% in males and 23.5%, 39.0% and 43.2% in females exposed to 20, 40 and 80 mg/kg DBTL respectively.

Effect of different concentrations of DBTL on retention of memory in albino rats of various age and sex after seven days of acquisition of memory, as assessed by CAR is shown in Fig. 43. As apparent from the figure, a dose dependent decrease in the retention of memory was noticed in all the groups of animals. Females of all the groups showed higher degree of decrease of CAR in comparison to the males of the same age group. Among the various groups of animals exposed to DBTL, males and females both of the juvenile group showed maximum decrease in the retention of memory and it in the various groups was of the following order: Juvenile > Adult > Weanling.

Biogenic amines:

Effect of different doses of DBTL on the levels of biogenic amines (NA, DA and 5-HT) in whole brain of juvenile female albino rats is shown in Fig. 44. As evident from the figure, the levels of all the three amines decreased in the whole brain of treated rats, in a dose dependent manner. Maximum inhibition as compared to that of other amines was noted in DA levels, this was statistically significant in comparison to the controls.

Influence of various doses of DBTL on the regional brain catecholamines (NA & DA) and 5-HT contents in juvenile female albino rats is shown in Figures (45-47). Hypothalamus and frontal cortex appeared to be the most affected brain areas, as levels of all the three amines were significantly lowered. A significant decrease in the contents of NA and 5-HT was also noted in cerebellum and pons - medulla. Additionally, the levels of NA and DA were reduced in corpus striatum. A similar pattern of change for each amines were observed in both groups of DBTL exposed rats in localized brain area.

DISCUSSION:

Certain organotin compounds such as di-n-butyltin dichloride, diphenyltin hydroxides, diethyltin and dimethyltin are reported to affect the growth, food intake and caused anemia (190-193). Unpalatability of diet, due to mixing of organotin compounds in food grain has been suggested to be one of the factors for such effects. A reduced body weight gain, lethargic conditions, hindlimb weakness and swelling around the mouth area in the DBTL treated rats observed in the present study. These changes do not appear to occur due to unpalatability of the DBTL mixed diet, since, it was given by oral intubation. However, reduced food intake due to sluggish conditions of rats or low absorption of nutrients from gastrointestinal tract may be responsible for decreased body weight gain in DBTL exposed rats. Generalized illness of animals, muscular weakness and paralysis has been reported in the animals exposed to organotin compounds (194-196). Similar observations have also been reported in humans exposed to organotin compounds (197). High rate of mortality in the juvenile and adult rats could be due to the high rates of metabolism in their liver. The low rate of mortality in weanling rats could be due to lack of drug-metabolizing enzymes, absence of sex hormonal influences, or low sensitivity of the central nervous system (due to immaturity) in these rats (198-200). It has been demonstrated that the rate of metabolism of xenobiotics was lowest in weanling rats, intermediate in adult rats and highest in juvenile rats (201). Variation in the toxicity of chemicals has been reported by many workers (185-188). The high rate of mortality in females in comparison with the males of same age group suggests a greater sensitivity of females towards DBTL in comparison to the males.

A marked difference in the toxicity of chemicals between male and female has been reported (202-205), which could be due to the difference in the hormones in males and females and their action on the distribution of the organotin compounds.

Catecholamines and serotonin act as modulators of number of important behavioral functions, i.e. arousal, thermoregulation, sensory perception, emotional and aggressive behavior (206-209). Alterations in the levels of these amines due to exposure to drugs such as amphetamine, apomorphine, or neurotoxic chemicals, e.g. manganese, acrylamide, styrene, methylmethacrylate and organotins have been found to cause disturbances in these functions (210-214). The present study showed that DBTL, like other organotin compounds, affected the levels of DA, NA and 5-HT and also the spontaneous and drug induced motor activity and learning ability. The observation that though the spontaneous locomotor activity is decreased on exposure to DBTL and the drug induced motor activity in the treated animals is significantly less than the controls at all doses. The same degree of percentage increase in motor activity and amphetamine administration in control and DBTL treated rats suggest that the effect on motor activity is more due to the muscular weakness than the sensitization of the CNS receptors. However, the role of CNS can not be ruled out since the levels of biogenic amines were altered significantly in the treated animals.

The biochemical study suggests that the frontal cortex and hypothalamus were the most affected brain area by DBTL since they showed a decrease in all these amines at 40 and 80 mg/kg doses, and corpus striatum, ponsmedulla and cerebellum, showed decrease only in NA and 5-HT. Although there was no uniform pattern

in regional changes in these amines, the magnitude of these alterations exhibited a dose-dependent effect. This could be due to the variations in the chemobiodynamics of DBTL in discrete brain areas. A similar pattern of changes in biogenic amines has been reported in animals exposed to acrylamide and dimethyltin (215-216). The cell bodies containing NA and DA are also localized in distinct neuronal pathways, the fibres of which innervate and terminate in discrete brain parts. Our observation of significant reduction in brain biogenic amines in selected brain areas may partly account for the observed behavioral changes in DBTL treated rats. These observations clearly indicated that exposure of rats to DBTL can lead to neurobehavioral and biochemical dysfunctions, which may be more pronounced in the juvenile rats specially the females.

Table - 45: Alterations in the body weight of 4 weeks old rats exposed to 0, 20, 40 or 80 mg/kg DBTL orally for three consecutive days.

DAYS	Male				Female			
	0mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	0mg/kg	20 mg/kg	40mg/kg	80mg/kg
I.	37	40	39	36	32	35	34	33
III.	43	44	42	39	38	39	36	35
V	48	47	44	40	45	42	37	36
VII.	54	52	45	42	50	45	37	37

* Values are the average body weight of 10 rats.

Table - 46: Alterations in the body weight of 8 weeks old rats exposed to 0, 20, 40 or 80 mg/kg DBTL, orally for three consecutive days.

DAYS	Male				Female			
	0 mg/kg	20 mg/kg	40mg/kg	80 mg/kg	0mg/kg	20mg/kg	40mg/kh	80mg/kg
I.	120	110	116	115	100	105	108	110
III.	126	114	118	117	106	108	110	111
V.	133	118	119	119	113	109	111	112
VII.	140	121	120	120	119	111	111	112

* Values are the average body weight of 10 rats.

Table 47 : Alterations in the body weight of 24 weeks old rats exposed to 0, 20, 40 or 80 mg/kg DBTL orally for three consecutive days.

DAYS	Male				Female			
	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg
I.	350	356	358	360	295	290	295	296
III.	356	362	360	361	300	294	297	297
V.	362	365	362	363	306	297	299	299
VII.	370	369	364	365	310	299	300	300

* Values are the average body weight of 10 rats.

Table - 48: Percent Mortality* as a result of exposure of various doses (20, 40 & 80 mg/kg) of DBTL on the albino rats of different age and sex.

	CONTROL RATS		DBTL EXPOSED RATS					
			10 mg/kg		40 mg/kg		80 mg/kg	
AGE OF ANIMLS	Male	Female	Male	Female	Male	Female	Male	Female
Weanling (4 weeks)	0.0%	0.0%	0.0%	0.0%	10%	20%	20%	30%
Juvenile (8 weeks)	0.0%	0.0%	0.0%	0.0%	10%	20%	30%	30%
Adult (24 weeks)	0.0%	0.0%	10%	20%	40%	60%	60%	90%

* Each group was having 10 animals.

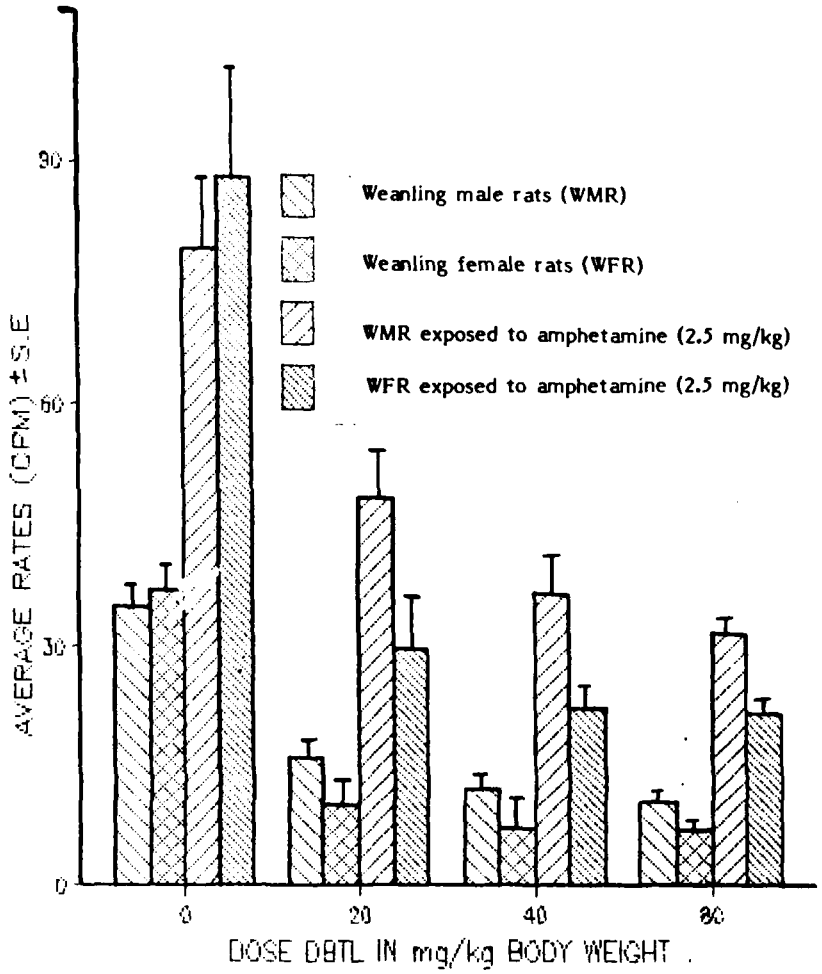


Figure - 37: Effect of different concentrations of DBTL on spontaneous and drug (amphetamine) induced motor activity in weanling male and female albino rats.

: Values are the average activity counts/minute ± S.E. of eight rats.

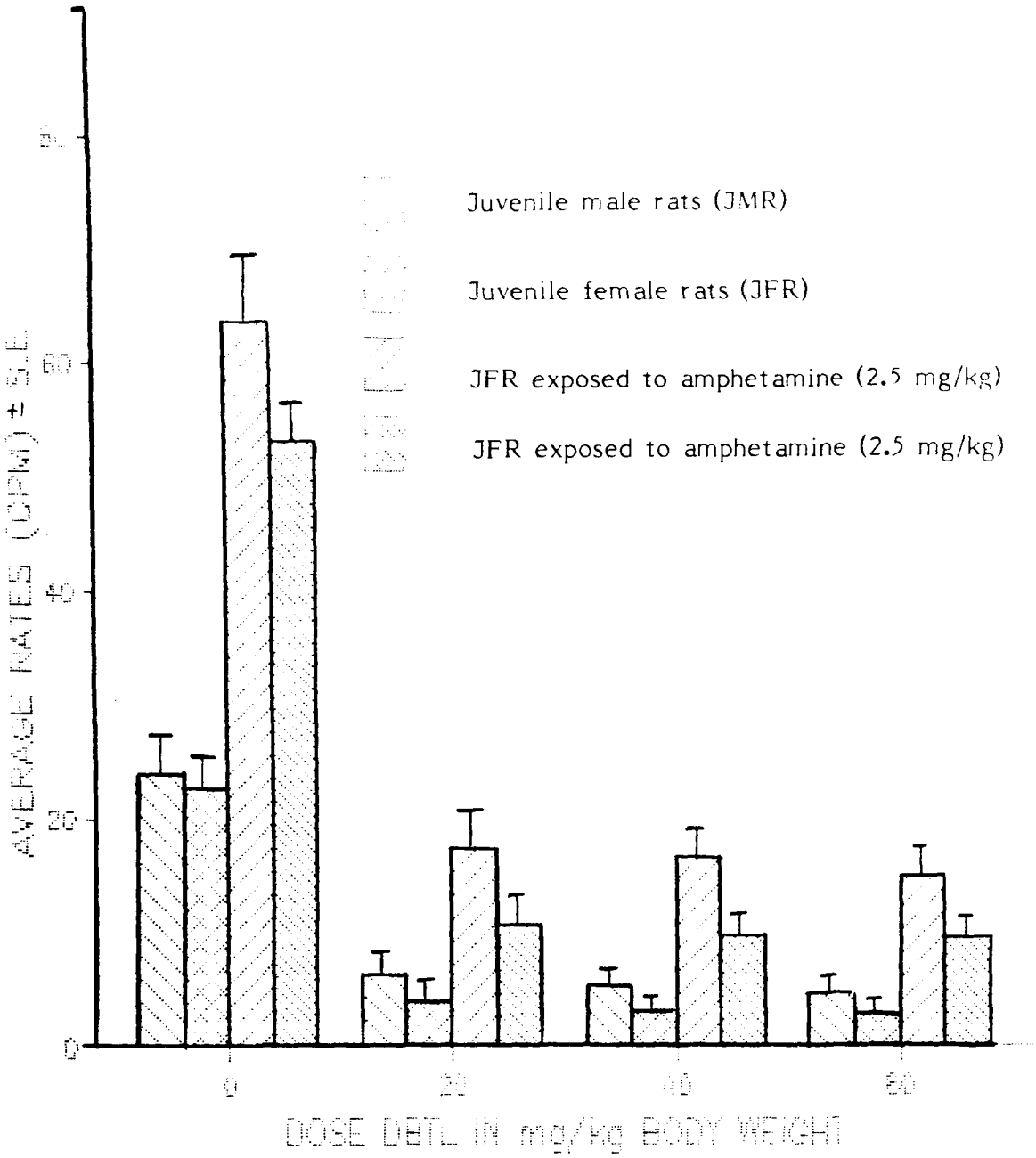


Figure - 38 : Effect of different concentrations of DBTL on spontaneous and drug (amphetamine) induced motor activity in juvenile male and female albino rats.

: Values are the average activity counts/minute ± S.E. of eight rats.

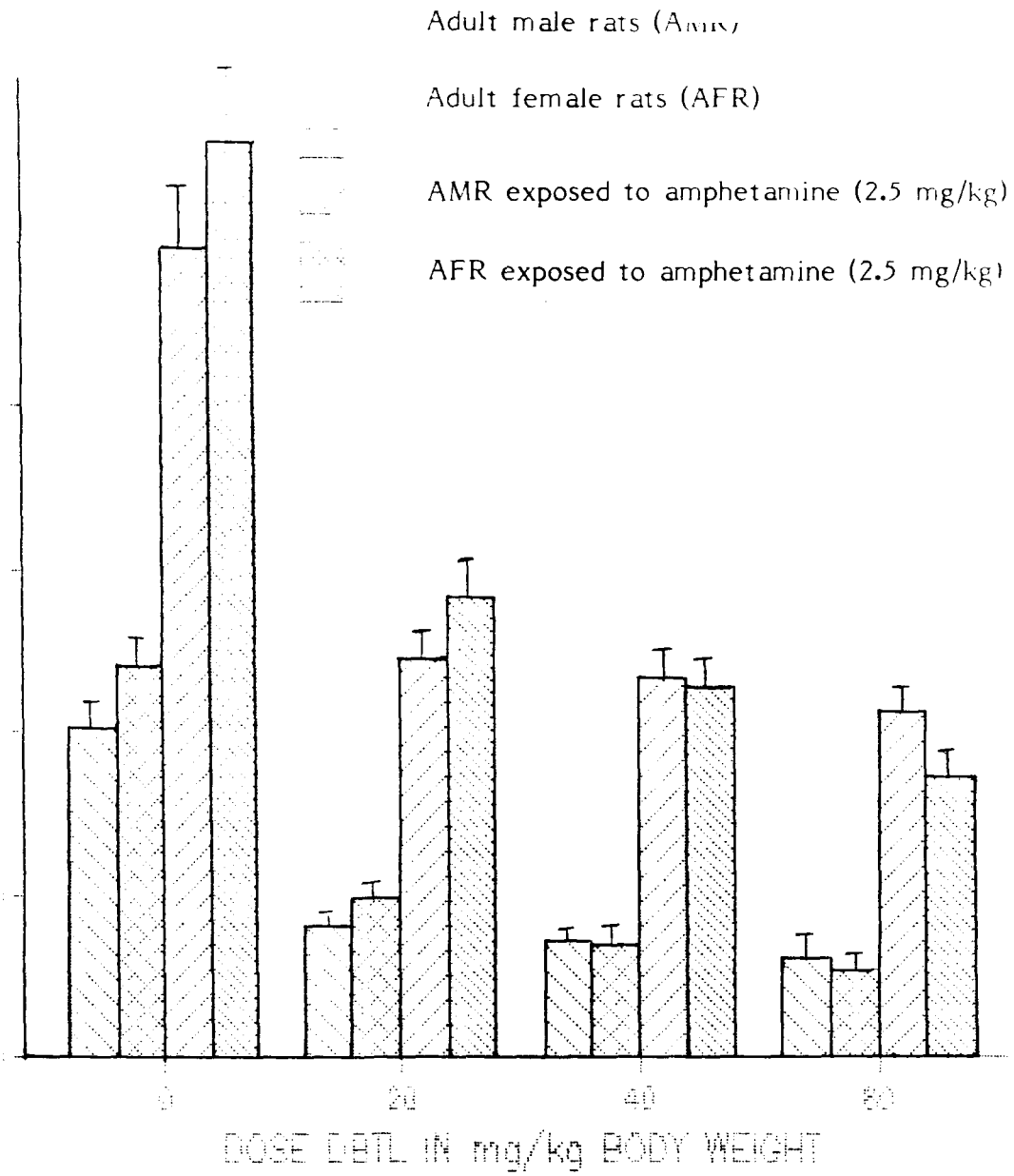


Figure - 39 : Effect of different concentrations of DBTL on spontaneous and drug (amphetamine) induced motor activity in adult male and female albino rats.

: Values are the average activity counts/minute ± S.E. of eight rats.

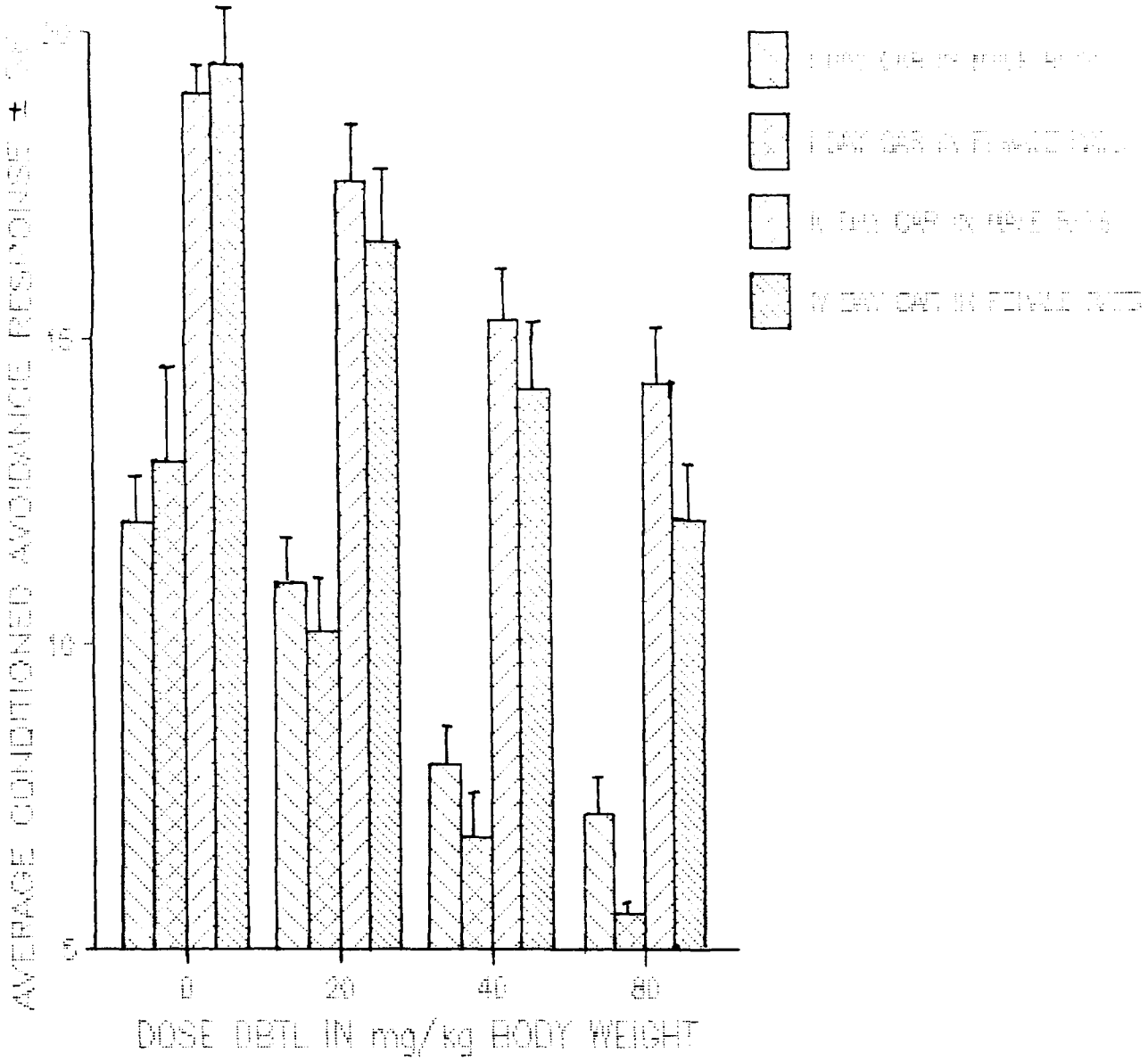


Figure - 40 : Effect of different concentrations of DBTL on learning ability of weanling male and female albino rats as assessed by conditioned avoidance response (CAR).

: Values are the average CAR ± S.E. of eight rats.

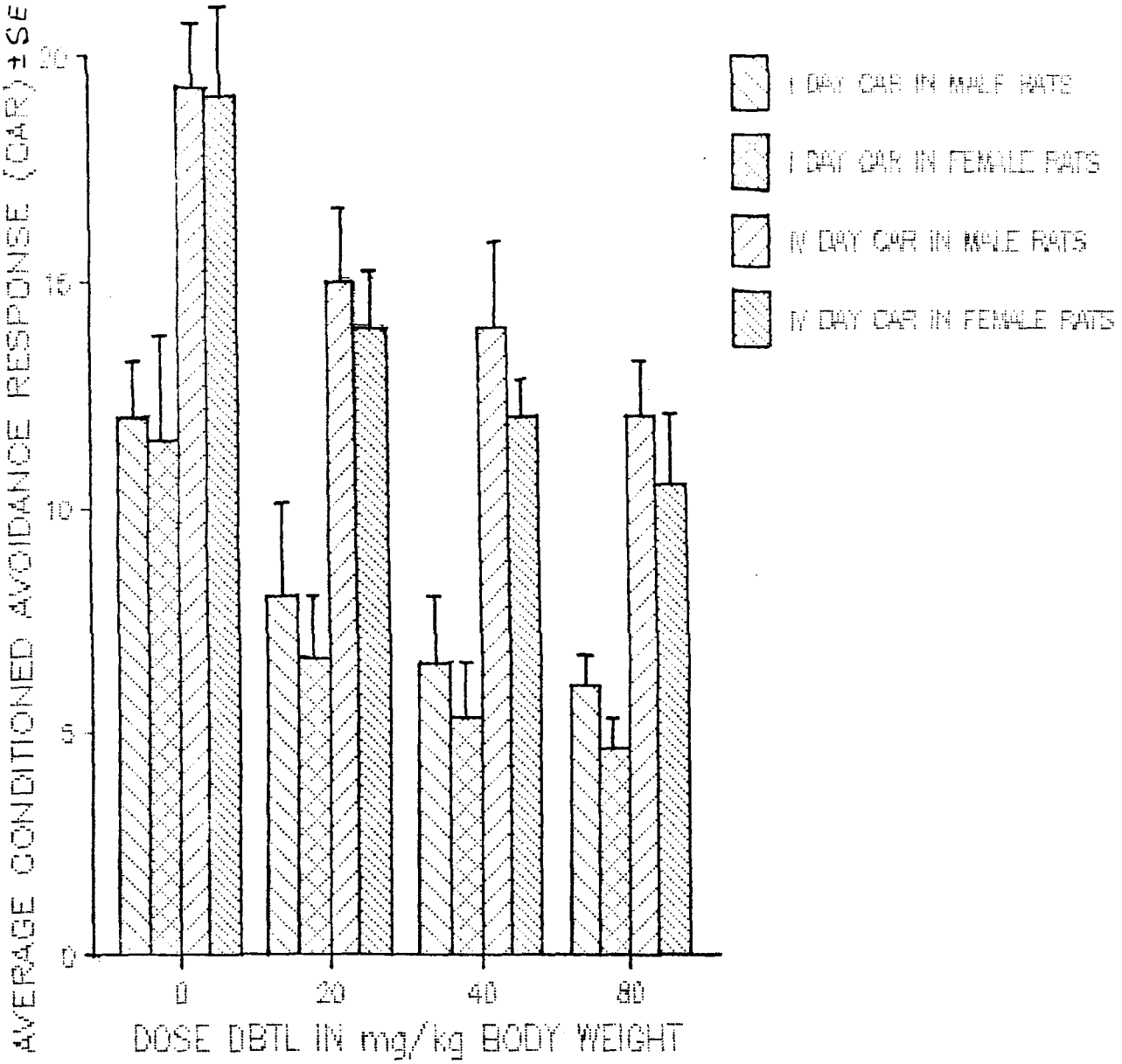


Figure - 41: Effect of different concentrations of DBTL on learning ability of juvenile male and female albino rats as assessed by conditioned avoidance response (CAR).

: Values are the average CAR ± S.E. of eight rats.

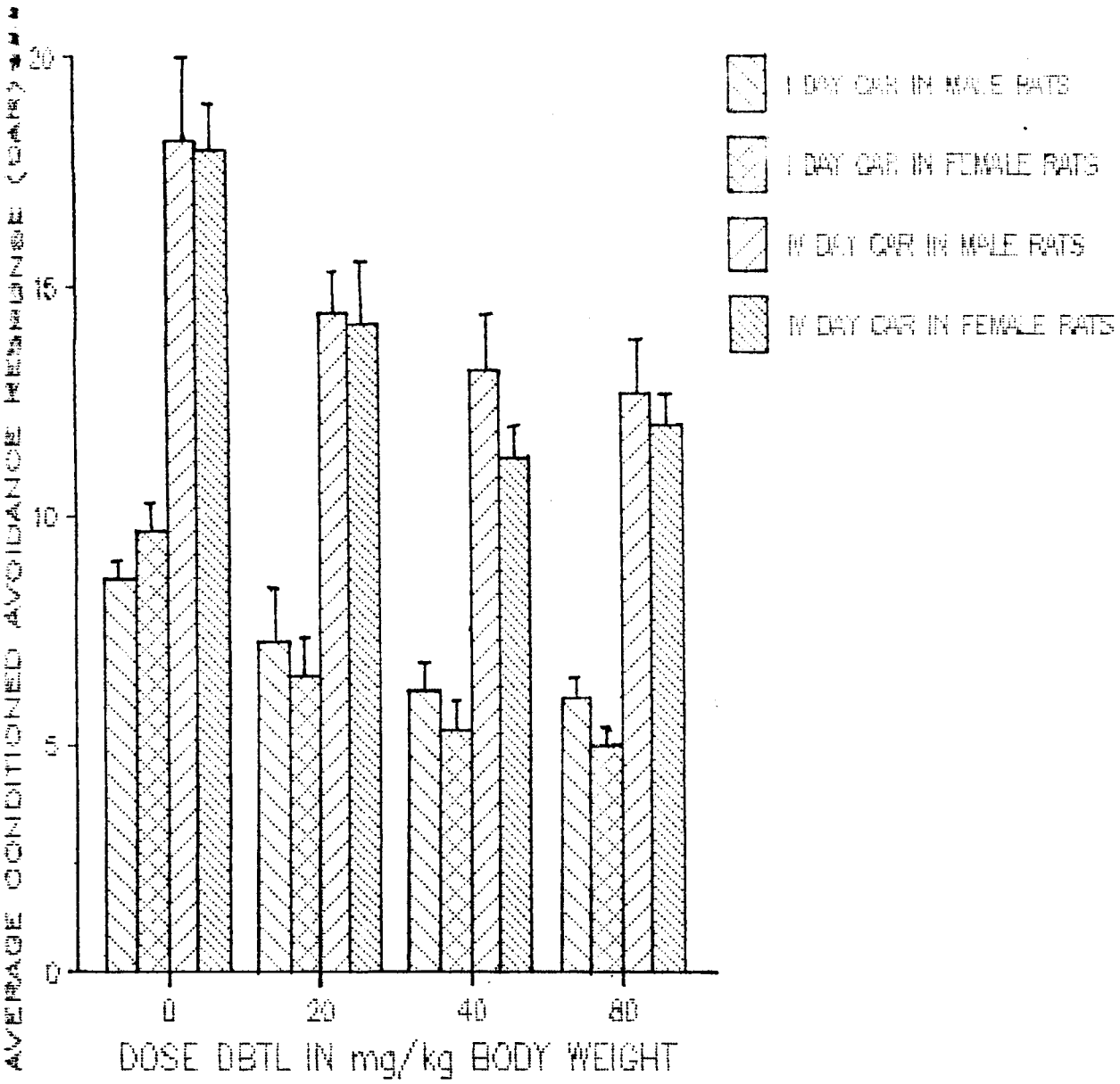


Figure - 42 : Effect of different concentrations of DBTL on learning ability of adult male and female albino rats as assessed by conditioned avoidance response (CAR).

: Values are the average CAR ± S.E. of eight rats.

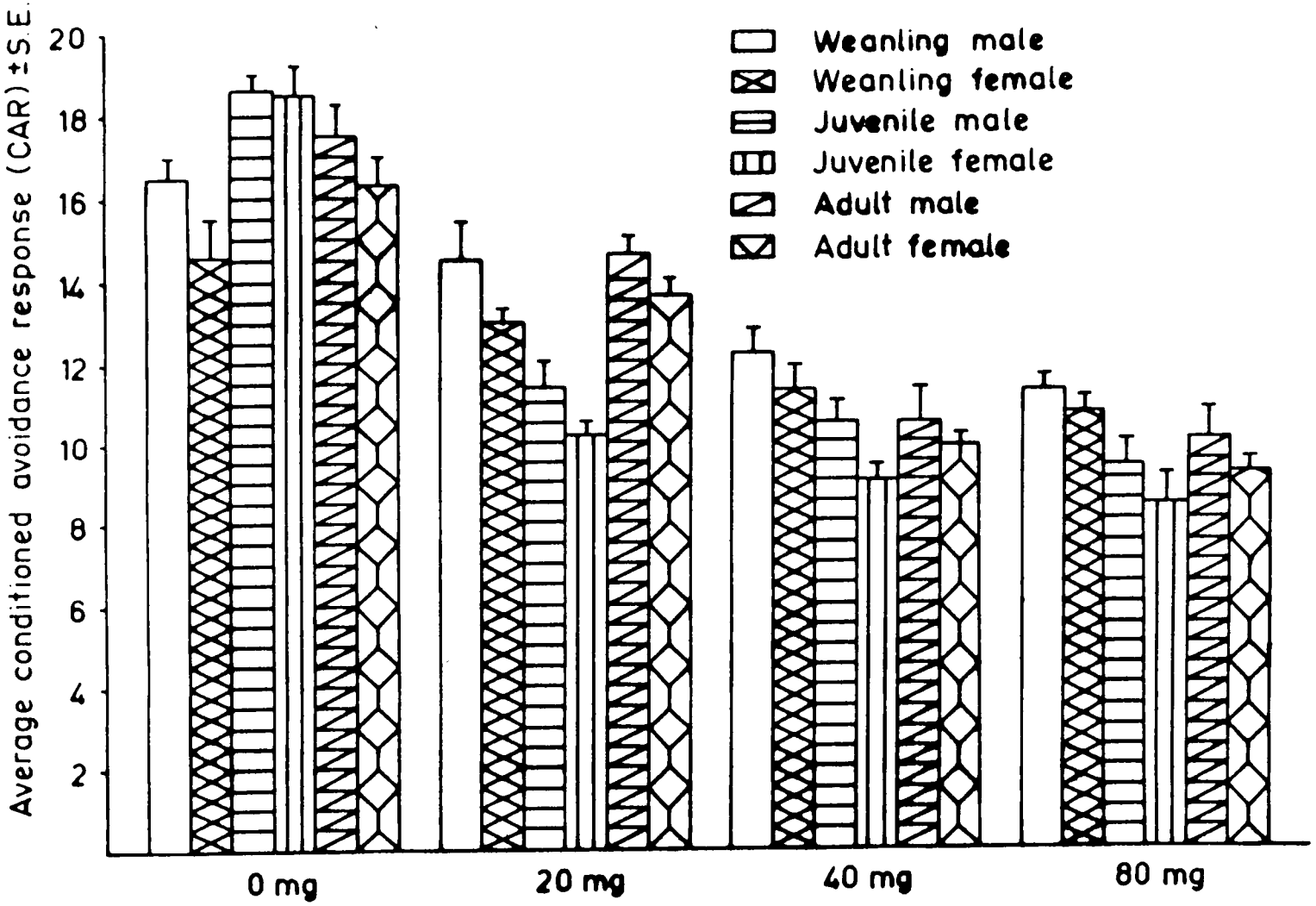


Fig:4.3: Effect of different concentrations of DBTL on retention of memory in the albino rats of virous age and sex, after 7 days of acquition of learning as assessed by average CAR ±S.E. of six rats, *$p<0.05$ (student's t. test)

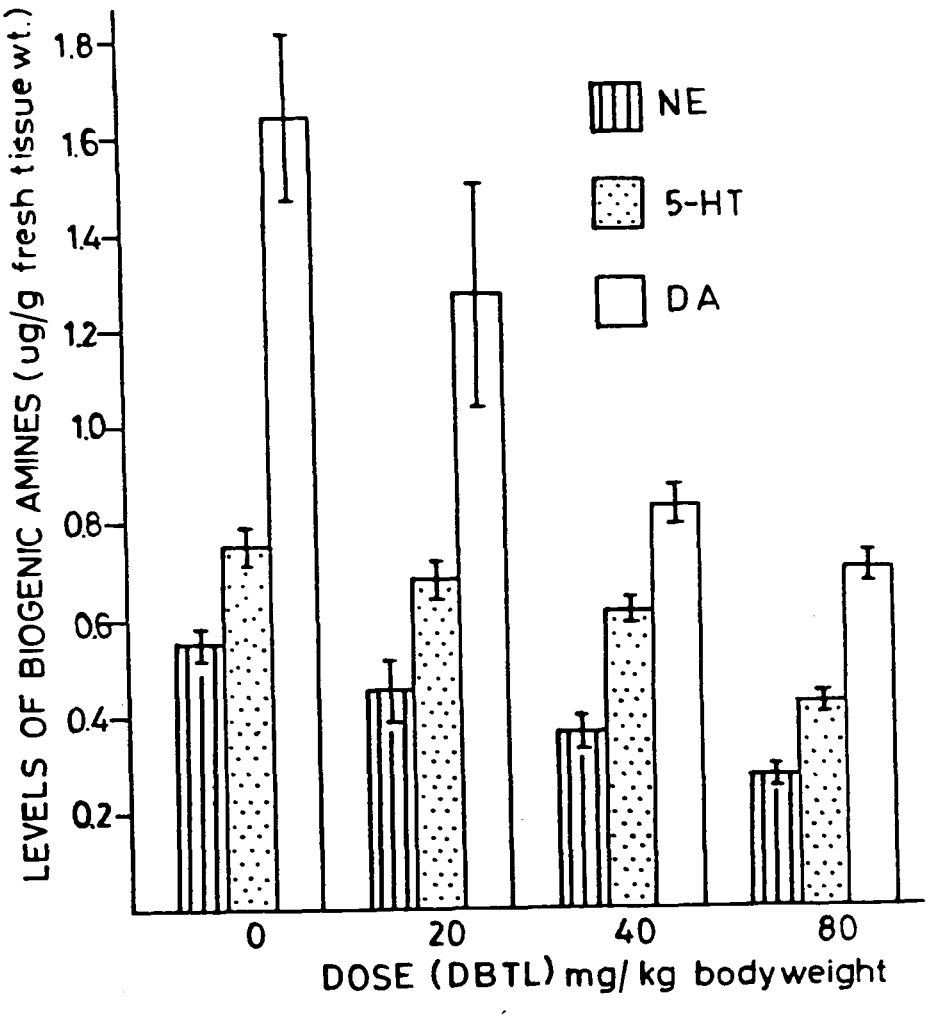


Fig- 44: Effect of different concentrations of DBTL on the levels of norepinephrine (NE) dopamine (DA) & 5-Hydroxy tryptaamine (5-HT) in female albino rats exposed orally for 3 consecutive days.

: Values represent in ug/gm fresh tissue weight ± S.E. of six rats, X 0.01 (student's 't' test).

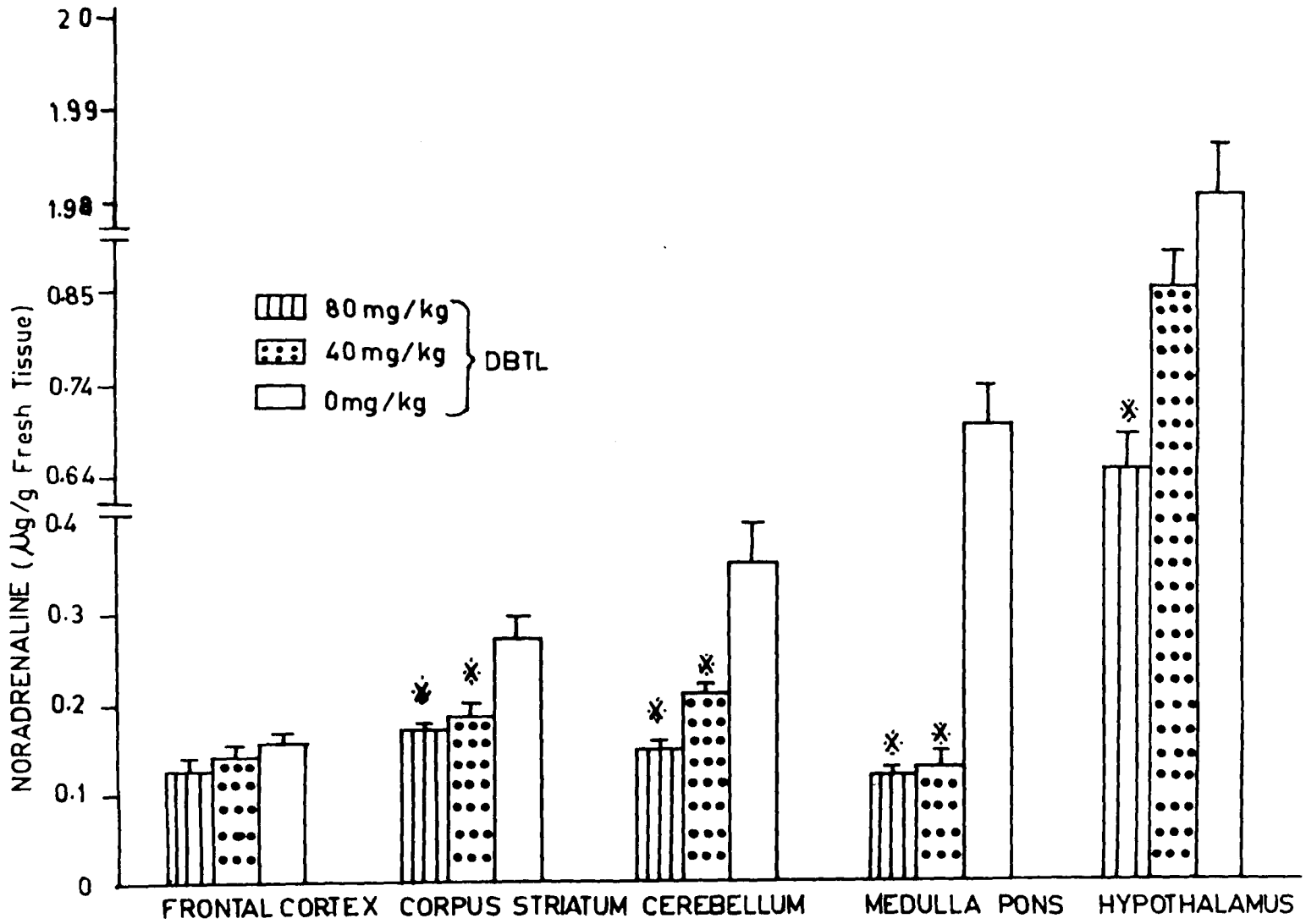


FIG.-45: Effect of different concentrations of DBTL on the levels of noradrenaline in different brain areas of female albino rats. Values represent the mean ± SE of six rats. * $p \leq 0.05$ (Student's t test)

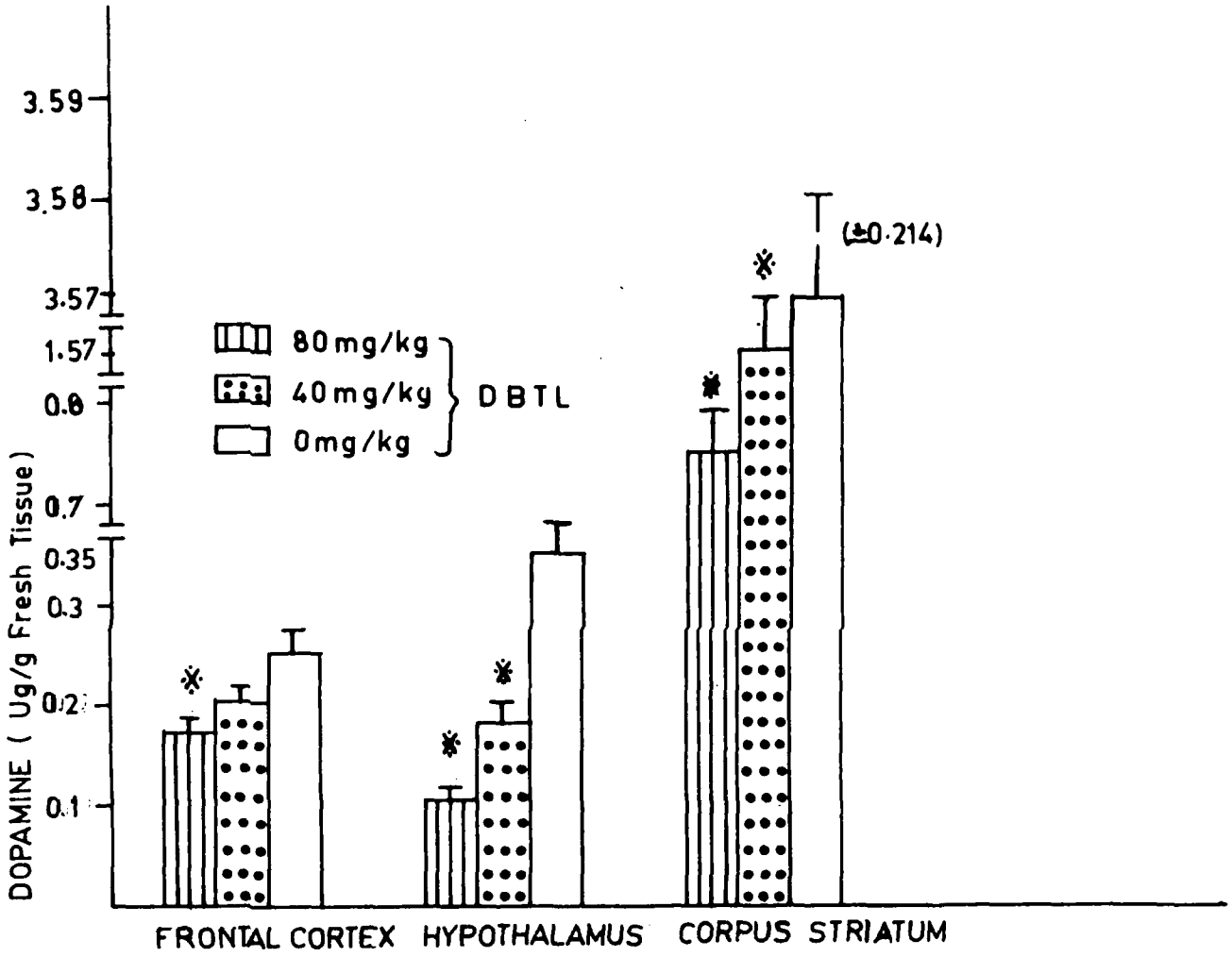


FIG.-46: Effect of different concentrations of DBTL on the levels of dopamine in different brain areas of female albino rats. Values represent the mean ± SE of six rats. * $p \leq 0.05$ (Student's t test)

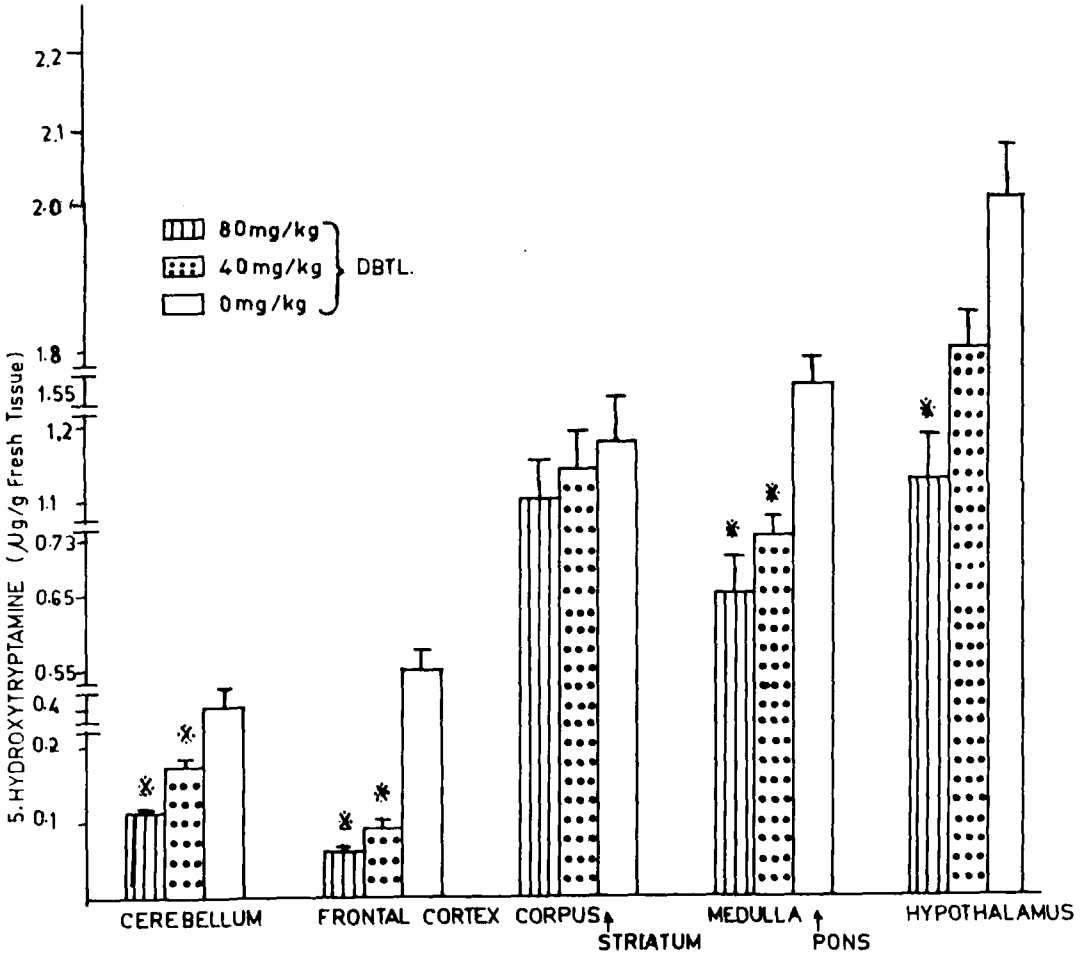


FIG.-47: Effect of different concentrations of DBTL on the levels of 5-hydroxytryptamine in different brain areas of female albino rats. Values represent the mean ± SE of six rats. * p/__. 5 (Student's t test)

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